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NITROGEN CYCLING THROUGH SOIL-PLANT SYSTEMS ON HIGH
ELEVATION RECLAIMED MINE SITES



by
HELEN FYLES

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF Master of Science

Soil Science

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THE UNIVERSITY OF ALBERTA
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled NITROGEN CYCLING THROUGH SOIL-PLANT SYSTEMS ON HIGH ELEVATION RECLAIMED MINE SITES submitted by HELEN FYLES in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The long term objective of reclamation practices at many mines in the Rocky Mountains of British Columbia is to create self-sustaining grassland systems capable of supporting wildlife. Such an objective requires that N recycling through soil and plants be sufficient to maintain plant productivity without continued additions of fertilizer.

The developmental sequence of a system following revegetation was examined using three reclaimed sites (2, 5, and 9 years old), seeded to grasses, and a native grassland. The flow of ^{15}N through soil and plant components was followed after addition of labelled $(\text{NH}_4)_2\text{SO}_4$. Nitrogen cycling was described in terms of: distribution of N among plant components, turnover of N in surface litter and roots, proportion of total N derived from labelled N in plants and mineralization of soil N.

The turnover times of N in surface litter and roots were about one and two years respectively. Nitrogen recycled through these components at the two oldest reclaimed sites and the native grassland, had created a pool of readily available N in the soil. The capacity of this active pool to mineralize N increased with site age and coincided with a decrease in the proportion of plant N derived from labelled N. Current reclamation practices at Westar Mining Ltd. produce grassland systems which recycle sufficient N to supply most of the N required to sustain present plant

productivity. Long term stability of such systems requires that the capacity of the active pool of N to mineralize N does not decline.

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I. INTRODUCTION

The long term objective of the reclamation program of many mines in the Rocky Mountains of British Columbia, is to create habitat suitable for wildlife consisting of approximately 30% tree cover and 70% grassland. If this objective is to be accomplished, a self-sustaining grassland system, a system that will survive without nutrient addition through fertilization, must be created. The establishment of a functioning grassland on the reclaimed mine spoil requires the development of a N cycle which involves both the retention and efficient cycling of N within the system. The present study was initiated to measure the accumulation of N in reclaimed mine spoil over a nine year period, and assess its potential to support plant growth without further inputs of fertilizer N. To achieve this objective, distribution and cycling of nitrogen within the soil/plant system were examined (Figure 1).

The potential of current reclamation practices to create self-sustaining grassland systems on subalpine sites, using agronomic species, has been questioned. The major concern is that fertilization produces high yields of grasses which result in a very thick litter layer on the soil surface the following year. It is thought that litter decomposition is slow thereby retarding transfer of N from litter into soil and effectively blocking the N cycle. Continued annual applications of fertilizer would therefore be necessary to provide N for plant growth. Such concerns

were the basis for the first objective of the study:

1. Examine the distribution of N among components of the reclaimed soil/plant system to determine if N was accumulating in any one component.

Difficulties in measuring N accumulation in reclaimed mine spoil are inherent in spoil derived from many coal-bearing formations. There are very high amounts of N in the sedimentary rock which makes up the raw spoil and these baseline levels mask the quantity of N that has accumulated since site establishment. Measurement of total soil N is essentially meaningless in such reclaimed mine spoils. It is however, possible to measure N mineralized in these soils as an index of N availability. The second objective of the study was, therefore, to:

2. Measure the potential of the revegetated reclaimed soils to mineralize nitrogen using a laboratory incubation technique.

Recycling of N in such soil/plant systems can be assessed indirectly by determining to what extent reclaimed sites are dependent on fertilizer to sustain their productivity each growing season. Labelled N can be used to distinguish recently applied fertilizer from N that has built up in the soil through fertilization and nutrient cycling.

Specifically, the objective was to:

3. Determine the proportion of N in plants growing on reclaimed mine spoil that was derived from fertilizer and the proportion that was derived from soil N accumulated over the years since site establishment.

The use of a labelled fertilizer allowed determination of the proportion of annually applied fertilizer taken up into the plants, and the amount that was lost from the system.

In addition to the labelled fertilizer study, the importance of N_2 fixing bacteria associated with grass roots in reclaimed areas was examined. An absence of legumes on the high elevation reclaimed grasslands under study, indicated that N_2 fixation by grasses, even in small amounts, could become a relatively important input of N to the system once fertilization has ceased. The final objective was therefore to:

4. Study the importance of free-living, N_2 fixing bacteria associated with grass roots on reclaimed areas.

II. LITERATURE REVIEW

Numerous studies of the distribution and circulation of N in North American prairie grassland ecosystems have been published including Bokhari and Singh (1975), Clark (1977), Reuss and Innis (1977), McGill *et al.* (1981) and Risser and Parton (1982). This research provides the fundamental framework for studying N accumulation and cycling in reclaimed grasslands although differences between the old, self-sustaining prairie systems and the native and reclaimed subalpine systems under study, must be considered. Variations in climatic factors, topography, soil characteristics, site history (grazing and fertilization) and plant species, are reflected in the distribution and flow rates of N. Direct comparisons between different grassland systems must, therefore, recognize that such variations exist.

A. NITROGEN CYCLING IN NATIVE GRASSLANDS

Grassland ecosystems are commonly described by defining various soil and plant components, measuring their size and calculating nutrient flows between them. The components and processes to be examined in this study are described in Figure 1. Pertinent literature will be reviewed using this figure as an outline. For more comprehensive descriptions of grassland nutrient cycles, see McGill *et al.* (1981) and Woodmansee *et al.* (1981).

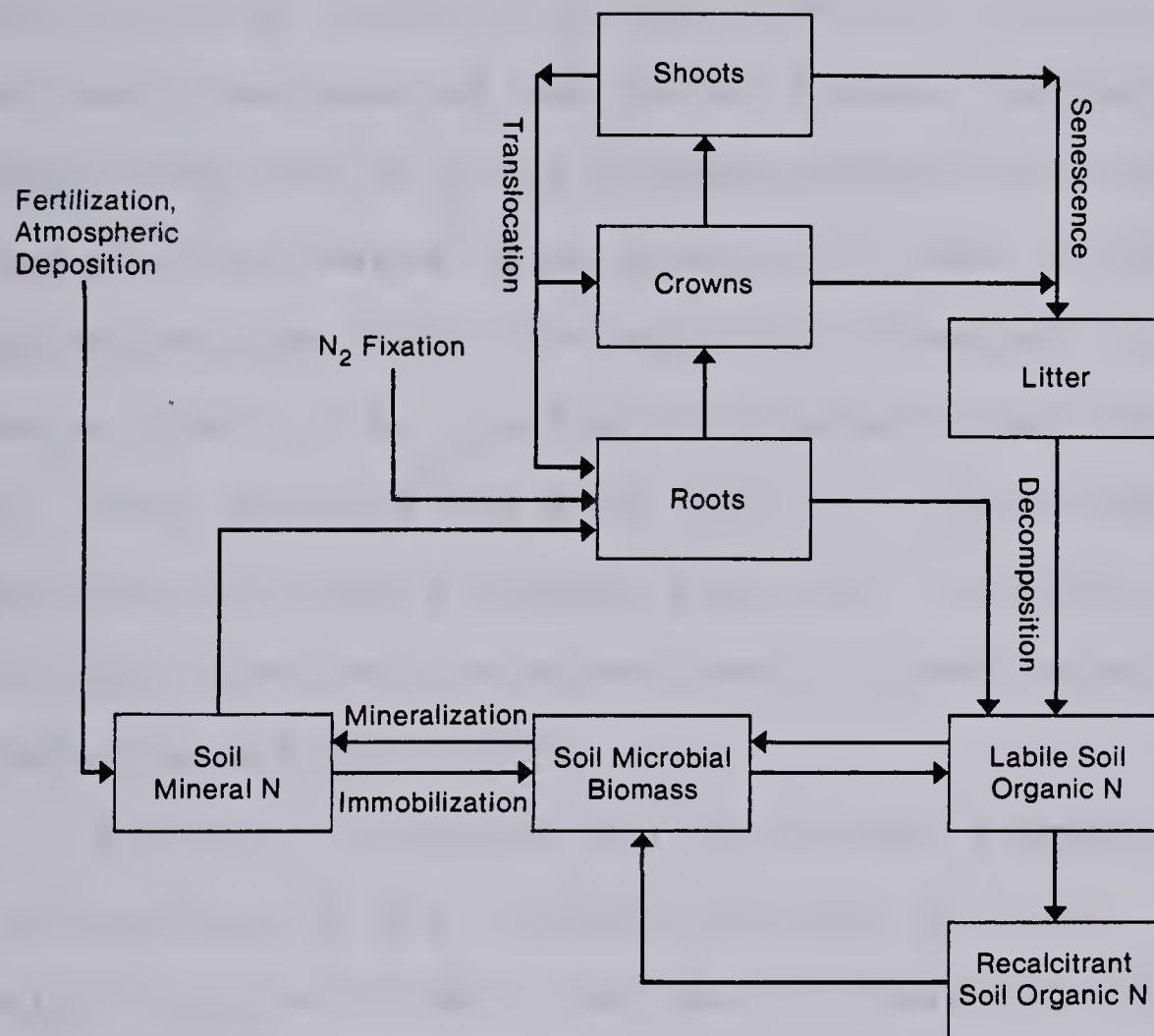


Fig. 1: Nitrogen cycling in grassland systems as described in this study.

NITROGEN INPUTS AND LOSSES

The principal inputs to unfertilized grasslands include N found in precipitation and fixation by free-living and symbiotic organisms (Reuss and Innis 1977). Estimations of precipitation inputs of N vary from 0.15 to 0.75 g/m²/y in various grasslands of the United States (Woodmansee 1978). Annual fixation of N₂ by legumes varies with location and year, and estimates range from 0.1 to over 10 g/m² (Jones and Woodmansee 1979). Non-symbiotic fixation in grasslands ranges from 0.2 to 0.4 g/m²/y (Vlassek *et al* 1973. Nelson *et al*. 1976, Kapustka and Rice 1978). In stable grassland systems such inputs from N₂ fixation, although small, offset N losses from the system and sustain plant productivity (Kapustka and Rice 1978).

Potential losses of N from natural grassland systems are leaching of NO₃⁻ through the soil profile, volatilization of NH₄⁺, and denitrification. These losses are insignificant in comparison to the amount of N which can be removed by grazing animals (Woodmansee 1978). Grazing removals are reviewed by Jones and Woodmansee (1979), and will not be covered here because effects of grazing are nonexistent in this study.

DISTRIBUTION OF NITROGEN WITHIN THE SYSTEM

Biomass data from different grassland ecosystems are summarized in Table 1. High elevation grasslands tend to have large shoot and root biomass and low levels of litter

TABLE 1. BIOMASS OF PLANT COMPONENTS IN VARIOUS GRASSLAND ECOSYSTEMS(g/m²)

| SITE | GRASSLAND TYPE | SHOOTS | LITTER | CROWNS | ROOTS (depth, cm) | REFERENCE |
|--------------|-----------------------|---------|---------|---------|----------------------|-----------------------|
| Dickinson | mixedgrass prairie | 178 | 689 | N.D. | 1263 (30) | Sims et al (1978) |
| Cottonwood | mixedgrass prairie | 87-95 | 394-563 | 212-258 | 803-1164 (30) | Sims et al (1978) |
| Hays | mixedgrass prairie | 79 | 750 | N.D. | 1150 (15) | Sims et al (1978) |
| Osage | tallgrass prairie | 141-173 | 535-804 | 136-195 | 658-1034 (50) | Sims et al (1978) |
| Pawnee | shortgrass prairie | 63-89 | 284 | 258-340 | 595-1043 (10) | Sims et al (1978) |
| Bridger | montane grassland | 121-241 | 107 | N.D. | 1388 (30) | Sims et al (1978) |
| Llyn Llydaw | montane grassland | 220 | 321 | N.D. | 603 (30) | Perkins (1978) |
| Sparwood | montane grassland | 100-150 | 475-525 | N.D. | 3200-3700 (24) | Ziemkiewicz (1982) |
| Medicine Bow | alpine meadow | 60-150 | 10-40 | N.D. | 3075 (17) | Thilenius (1975) |

accumulation relative to the other grasslands studied, indicating a high rate of litter decomposition in these sites.

Data on the distribution of N within high elevation grassland systems are scarce. Ziemkiewicz (1979) described the distribution of N within a subalpine grassland over two growing seasons. Characteristically, the majority of the N in the system (over 90%), was in the soil. Of the N in the plant components, the roots contained between 65 and 82% and the litter between 18 and 28% at peak standing crop.

Data from three prairie systems are summarized in Table 2 for comparative purposes. Distribution of N within the plant components varied with grassland type. On the shortgrass prairie almost half the plant N was in the roots and about 25% in the litter while on the tallgrass prairie, the major proportion of plant N was in the litter (43%), and only 32% was in the roots. The crowns were a more important component of the shortgrass prairie than in the other two systems. The high annual variation evident in Table 2 is common to natural grassland systems (Thilenius 1975) and should be considered when general conclusions are drawn from only one or two years of data.

DYNAMICS OF GRASSLAND COMPONENTS

Roots and crowns are the dominant living plant components in perennial grasslands (Woodmansee *et al.* 1981). At peak standing crop, the mass of live roots is 2 to 10

Table 2. Distribution of nitrogen in the plant components of three grassland types¹

| SITE | YEAR | SHOOTS | LITTER | CROWNS | ROOTS | TOTAL PLANT N |
|-----------------------------|------|--------|--------|--------|-------|---------------------|
| (% of total plant nitrogen) | | | | | | (g/m ²) |
| Pawnee | 1970 | 11.1 | 17.4 | 14.8 | 56.7 | 12.9 |
| | 1971 | 3.7 | 28.7 | 22.9 | 44.6 | 8.4 |
| | 1972 | 9.7 | 29.4 | 23.1 | 37.8 | 9.1 |
| Cotton -wood | 1970 | 9.9 | 42.7 | 15.1 | 32.3 | 17.2 |
| | 1971 | 10.1 | 38.5 | 1.6 | 49.9 | 15.4 |
| | 1972 | 10.5 | 32.1 | 12.0 | 45.4 | 20.1 |
| Osage | 1970 | 6.5 | 37.1 | 18.5 | 37.9 | 9.6 |
| | 1971 | 14.1 | 44.7 | 6.4 | 34.8 | 12.4 |
| | 1972 | 16.5 | 48.6 | 11.9 | 23.0 | 18.2 |

Table 3. Nitrogen transfers in three grassland systems (g/m²/y)²

| | PAWNEE | COTTONWOOD | OSAGE |
|-----------------------------------|-----------|------------|-----------|
| N release from litter | 0.47-1.71 | 2.11-3.55 | 0.30-5.13 |
| N release from roots+crowns | 2.64-5.87 | 1.14-4.37 | 1.59-4.30 |

1. Calculated from Bokhari and Singh(1975)

2. Bokhari and Singh (1975)

times as large as that of the shoots (Clark 1977, Sims *et al.* 1978), with most roots found in the upper 15 cm of the soil (Holechek 1982). Root death occurs during periods of desiccation and near the end of the growing season when carbohydrates available for downward translocation are reduced by demands of the flowering plants (Ares 1976).

The decomposition rate of root material is related to its C:N ratio and lignin and carbohydrate content (Power 1968, Herman *et al.* 1977). On the basis of C:N ratio, McGill *et al.* (1981) divided decomposing root material into a structural component which decomposes slowly, and a cytoplasmic or rapidly decomposing component. Decomposition is ultimately controlled by soil temperature and moisture content (Ares 1976, Singh and Gupta 1977, Sims and Singh 1978).

Annual turnover of roots, estimated in several grassland types, ranged from 25% in a mesic tallgrass prairie (Dahlman and Kucera 1965) to 49% in a xeric, shortgrass prairie (Sims and Singh 1978). These turnover times, determined for the entire spectrum of root materials give only an average value because the turnover of fine roots is faster than that of mature coarse roots (Ares 1976, Clark 1977).

Bokhari and Singh (1975) estimated the amount of N released from roots and crowns over three growing seasons and in three grassland types (Table 3). It is evident from these data that although variability is high, substantial

amounts of N are cycled through these components annually.

The size of the litter component on the soil surface is dependent on shoot production in previous years and rate of litter decomposition. Over the long term, in stable systems, litter losses through natural decay processes are balanced by seasonal increments of shoot material (McFadyen 1964). Factors affecting litter decomposition are similar to those listed for roots. The average annual turnover in a tallgrass prairie was estimated at 25% by Sims and Singh (1978), while other values for temperate grassland communities ranged from 0.14 to 0.50% per day (Singh and Gupta 1977). Litter in a montane grassland in Snowdonia, U.K., decomposed at a rate of 0.34% per day (Perkins 1978). The amount of N released from litter on three prairie grasslands reflected the varying size of the litter component (Table 3). On the shortgrass prairie, N recycled by litter may amount to 1.7 g/m²/y compared to over 5 g/m²/y in the tallgrass prairie.

In high elevation regions, a substantial amount of litter decomposition occurs in winter under snow. Litter bag studies in subalpine and alpine meadows, demonstrated that 28 to 35% of the litter present at the onset of winter decomposed under snow and represented 70 to 80 % of the litter decomposed annually (Bleak 1970, Knight and Kyte 1975). Once sufficient snow-pack has accumulated to buffer the soil surface from extremes of air temperature, the temperature of the litter zone rises above 0°C, and decomposing organisms can initiate activity (Bleak 1970,

McBrayer and Cromack 1980, Aitchison 1983).

Translocation of N from shoots to crowns, roots and rhizomes during late summer and fall is an important, but poorly documented, N recycling process (Perry and Moser 1974, McKendrick *et al.* 1975, Dickinson 1983). On a short grass prairie, the proportion of shoot N translocated before senescence, ranged from 9 to 54% with an average of 33% (Clark 1977).

Most N in natural grassland systems is in organic form in the soil. Of this soil organic N, 10-15% is in an active or readily mineralizable fraction and the remainder is in a stable, slowly mineralizable fraction (Jansson 1975). The processes of N immobilization and mineralization to and from these fractions are major controls on the size of the mineral N pool. Characteristically, grassland soils have low concentrations of NH_4^+ (<10 ppm) and NO_3^- (<1 ppm) because uptake capabilities of microorganisms and plants together exceed N mineralization (Woodmansee *et al.* 1981). In these systems, measurement of the potential of soil to mineralize N provides more information about the system than measuring the size of the mineral pool at any one time.

The microbial biomass, which decomposes soil organic matter, plant residues and dead microorganisms, is responsible for mineralizing and immobilizing a large proportion of N cycled in soil. Woodmansee *et al.* (1981) calculated that bacterial and fungal N represented 1% of total N in the system. Rapid microbial turnover, estimated

at 5.5 times per year (McGill *et al.* 1981), magnifies the contribution of this component to N flow through the system.

The effect of soil fauna on N cycling, through fragmentation and mixing of organic material and predation of primary saprophages, such as bacteria, fungi and actinomycetes, is well-documented (Anderson *et al.* 1981, Elliot *et al.* 1983) but is not examined in this work.

B. ECOSYSTEM DEVELOPMENT ON RECLAIMED MINE SPOIL

Recent research on minespoil reclamation has been concerned with comparing the size and turnover of some components of the developing ecosystem to adjacent undisturbed or cultivated systems. Root and shoot growth, litter accumulation and decomposition, levels of total N, N mineralization, microbial activity and N₂ fixation have all been examined in some detail in various mine spoils throughout the world, but little effort has been made in any one location to quantify the total contribution of all processes to N cycling within the revegetated spoil.

NITROGEN INPUTS AND LOSSES

Ecosystems developing on reclaimed mine wastes have very low initial levels of N, and inputs occur through a combination of atmospheric deposition, fertilization and N₂ fixation. Atmospheric deposition in reclaimed systems is similar to that for natural systems and is related to geographic location and rainfall. Marrs *et al.* (1980)

calculated an annual addition of 9 kg/ha to reclaimed china clay wastes in Cornwall, and Dancer(1975) estimated 13 kg/ha for a similar location. The quantity of N added through fertilization varies with the management practices and is specific to each reclaimed area.

The importance of N₂ fixation by legumes for increasing soil N levels in reclaimed systems is well-documented (Dancer *et al.* 1977, Skeffington and Bradshaw 1980, Bradshaw 1983). Skeffington and Bradshaw (1980) demonstrated a potential fixation rate by *Trifolium repens* of 49 kg/ha/y on reclaimed china clay wastes in Cornwall. Nitrogen fixation rates exceeded 250 kg/ha/y for several legume cultivars grown on sand and mica wastes in Cornwall (Dancer *et al.* 1977).

The significance of non-symbiotic N fixers in reclaimed areas is not clear. In New Mexico, coal mine spoils, exposed for one year, and an undisturbed soil under sagebrush had similar numbers of N₂ fixing bacteria (Miller *et al.* 1979). In 14 year old spoil from the same area, the population of N₂ fixing bacteria was one tenth that found in the one year old spoil. In reclaimed mine spoil in North Dakota, Koponen *et al.* (1980) found between 53 and 71% of the bacteria isolated from the rhizosphere of grass plants were capable of reducing acetylene. The reclaimed site with no topsoil supported bacteria which were individually capable of reducing significantly higher amounts of acetylene than those isolated from other sites. Skeffington and

Bradshaw(1980) found that a *Festuca rubra-Agrostis tenuis* grassland fixed over 9 kg/ha/y under optimum field conditions. Dancer *et al.* (1977) estimated the amount of N fixed by non-symbiotic bacteria and other microorganisms to be less than 30 and 10 kg/ha/y for mica and sand wastes respectively.

Losses of N from reclaimed grasslands through leaching, volatilization and denitrification have been documented in only a few instances. Leaching losses were studied experimentally in lysimeters by Marrs and Bradshaw(1980). In recently revegetated waste dumps, 5 and 30 kg/ha/y of N were lost from the system. In older vegetated sites, losses of fertilizer were lower, between 1 and 12 kg/ha/y or 5% of applied N. Dancer *et al.* (1979), working on similar spoil, found that 10% of applied fertilizer was lost from revegetated spoil annually.

Potential losses of N by volatilization may occur after application of ammonium fertilizers when soil pH exceeds 7.0, but it is expected that these losses are low (Marrs and Bradshaw 1980). Denitrification is not considered to be significant in the freely draining conditions common to most mine spoil, but there are no data to confirm this.

ROOT AND SHOOT BIOMASS

Root biomass in three year old reclaimed mine spoil, adjacent to the area under study in this work, was approximately 400 g/m² to a depth of 24 cm (Ziemkiewicz

1982). This reclaimed site was not fertilized in the year prior to sampling. A similar fertilized site produced over 500 g/m² of roots and a nearby native grassland produced up to 7 times this amount. At peak standing crop, roots in the fertilized reclaimed site contained 4.6 g/m² of N compared to 2.6 g/m² in the same, unfertilized site. In fertilized and unfertilized sites, root N represented 27 and 47% of the total plant N respectively, suggesting that fertilization increased the importance of shoots and litter relative to roots as sinks for N.

Root biomass in a naturally revegetated, 40 year old mine spoil in Montana was 45.2 g/m² to a depth of 15 cm, and was comparable to that of native range (Holechek 1982). In the same study, the root biomass of a five year old, annually fertilized reclaimed site was 127 g/m².

Fertilization practices also strongly affect the size of the shoot biomass. For example, shoot production in the three year old reclaimed site described above, (Ziemkiewicz 1982), ranged from 150 g/m² on the unfertilized site to 400 g/m² on the fertilized site. These data indicate that management procedures can be manipulated to control the size of components within reclaimed grassland systems. Above and belowground productivity is therefore specific to each mine site and its particular management practices.

SURFACE LITTER ACCUMULATION

High shoot productivity in fertilized areas can result in a large litter component the following growing season. The accumulation of large amounts of litter on the surface of reclaimed mine spoil has been noted in several studies (Vimmerstedt and Finney 1973, Marrs *et al.* 1980, Schafer *et al.* 1980, Ziemkiewicz 1982). Litter can represent over 50% of total plant N (Ziemkiewicz 1979) and its decomposition is essential for recycling of N in reclaimed systems.

Fyles (1980) measured a surface litter accumulation rate of 53 g/m²/y in a high elevation reclaimed area adjacent to the sites studied in this work. Marrs *et al.* (1980) reported that N accumulated in surface litter on reclaimed china clay wastes (1 to 12 years old) in England. There was no correlation however, between site age and quantity of litter N and it was evident that factors influencing decomposition varied with site location, plant species and fertilization practices. Accumulation of leaf litter occurred on the surface of coal mine spoil in Ohio, which had been planted with hardwoods (Vimmerstedt and Finney 1973). Introduction of earthworms to the spoil resulted in the consumption or burial of 500 g/m² of litter and substantially reduced surface litter accumulation during a two year period.

Several studies have estimated litter decomposition on revegetated minespoil using litter bag experiments (Lawrey 1977b, Hutson 1980, Weider *et al.* 1983). Lawrey (1977b)

found that a 20 year old, naturally revegetated mine spoil and an adjacent, undisturbed area, had similar decomposition potentials. On a tallgrass prairie in central Missouri, 23% of the initial fescue litter remained undecomposed on a vegetated mine spoil after 20 months (Weider *et al.* 1983). This was similar to the proportion of litter decomposed on an undisturbed prairie soil and was significantly lower than the 40% remaining on the unvegetated mine spoil. The rate of N transfer from plant materials to soil and roots was calculated by Marrs *et al.* (1980), on clay waste tips in England. They reported that up to 35% of the N taken into plants was recycled annually in tips revegetated to grass and clover.

Decomposition rate or potential has also been assessed by measuring the weight loss of cellulose strips placed on the surface of reclaimed mine spoil. Very little cellulose decomposed until 2 months after placement on the surface of either an undisturbed grassland or a 30 year old reclaimed site in southern Alberta (Visser *et al.* 1983). Rapid decomposition in the disturbed site occurred after this time, and 44 months after initiation of the study, virtually all the cellulose had been utilized. After the same period on the undisturbed grassland, almost 72% of the cellulose remained. High cellulose utilization on the reclaimed site was a result of rainfall embedding the cellulose strips in the soil where greater contact with soil microorganisms and improved moisture and N status all contributed to rapid

decomposition. This suggested that although surface conditions on both the natural and reclaimed systems were not conducive to cellulose decomposition, decomposition proceeded rapidly once the cellulose was incorporated into the soil. A study by Weider *et al.* (1983) produced similar results to the southern Alberta study, with 14% of the cellulose remaining on the vegetated 10 year old mine spoil and 62% remaining on the undisturbed tallgrass prairie after 27 months. No explanation of these data is given. In the same study, 80% of the cellulose remained after 27 months on an unvegetated mine site.

Cellulose decomposition must be supported by indigenous N mineralization and it is therefore also used to estimate the availability of N in developing minespoil. Fyles (1980) found that the decomposition of cellulose strips, buried 5 to 10 cm below the surface was similar on a three year old fertilized, reclaimed site and an adjacent native grassland. Almost 20% of the cellulose decomposed in one growing season in each site. A six year old site, not fertilized in the year of study, showed only a 6% loss of cellulose in the same time period. This was attributed to the dependence of the reclaimed sites on N fertilization to provide sufficient mineral N to permit decomposition of high C:N ratio organic matter.

CARBON AND NITROGEN ACCUMULATION

The accumulation of C and N in revegetated spoil have been measured in spoils of varying age and origin (Table 4). Organic C and total N varied widely between locations but all studies reported an increase in %C and %N with age and a decrease with spoil depth. Rates of accumulation in naturally revegetated areas were measured in only two studies and ranged from 28.2 g/m²/y in southern Saskatchewan (Anderson 1977), to 45.4 g/m²/y in southeastern Montana (Schafer *et al.* 1980).

The significance of management practices to organic matter build-up in surface layers of spoil was demonstrated by Schafer *et al.* (1980). Very high rates of C and N accumulation, 134.9 and 7.9 g/m²/y respectively, were attributed to heavy fertilization, seeding with vigorous perennial grasses and alfalfa, and a lack of grazing. Low microbial activity in minesoils, (Hersman and Temple 1978), and dry exposed surface conditions, (Schafer *et al.* 1980), were also thought to contribute to C accumulation in the upper layer of the soil.

Roberts *et al.* (1980) measured accumulation of N in three components (shoots, roots and surface soil) in annually fertilized, seven year old reclaimed china clay waste. These authors found an overall N accumulation of 6.3 g/m²/y and measured a relatively higher accumulation in root and shoot components compared to the soil. High root N was attributed in part, to fixation of atmospheric N₂ by

TABLE 4. CARBON AND NITROGEN ACCUMULATION IN VARIOUS SPOIL TYPES

| LOCATION AND SPOIL TYPE | SPOIL AGE (years) | SPOIL DEPTH (cm) | ORGANIC C (%) | C ACCUM. RATE (g/m ² /y) | TOTAL N (%) | N ACCUM. RATE (g/m ² /y) | REFERENCE CITED |
|---------------------------------------|-------------------------|------------------------|---------------------|---|-------------------|---|------------------------------|
| Minnesota Ironstone spoil | 2 | 0-2.5 | 0.08 | N.D. | 0.005 | N.D. | Leisman (1957) |
| | | 7-10 | 0.06 | | 0.003 | | |
| | 51 | 0-2.5 | 1.26 | N.D. | 0.098 | N.D. | |
| | | 7-10 | 0.72 | | 0.060 | | |
| Somerset Colliery spoil | 5 | 0-2 | 0.87 | N.D. | 1.10 | N.D. | Down (1975) |
| | | 8-10 | 0.39 | | 1.00 | | |
| | 55 | 0-2 | 3.47 | N.D. | 1.30 | N.D. | |
| | | 8-10 | 1.12 | | 1.00 | | |
| Saskatchewan Glacial till spoil | 27 | 0-2.5 | 1.88 | 28.2 | 0.18 | 2.67 | Anderson (1977) |
| | | 5-10 | 0.41 | | 0.05 | 2.60 | |
| | 40 | 0-2.5 | 3.41 | 28.2 | 0.24 | 2.60 | |
| | | 5-10 | 0.37 | | 0.05 | | |
| Montana sand, siltstone spoil | 6 ² | 0-2.5 | 2.00 | 134.9 | 0.15 | 7.88 | Schafer et al. (1980) |
| | | 5-10 | 0.30 | | 0.02 | | |
| | 50 | 0-2.5 | 4.30 | 45.4 | 0.36 | 2.60 | |
| | | 5-10 | 1.10 | | 0.07 | | |
| Iowa | 100 | 0-5 | 2.60 | N.D. | N.D. | N.D. | Hallberg et al. (1978) |
| Loess spoil | | 5-10 | 1.50 | N.D. | N.D. | N.D. | |

1. Mean rate calculated from 4 revegetated sites
40, 28, 28, and 27 years old.

2. This 6 year old spoil was fertilized and seeded
with introduced grasses and shrubs.

legumes.

NITROGEN MINERALIZATION

The mineralization of organic N to NH_4^+ is an essential link in the N cycle. Incubation of reclaimed mine spoil under conditions which promote mineralization of organic N has frequently been used to assess the availability of soil N to plants (Williams 1975).

Net N mineralization after a 168 day incubation differed significantly between a five year old vegetated spoil dominated by alfalfa, and an undisturbed soil, from a high elevation site in Colorado (Reeder and Berg 1977b). Net mineralization was 49 and 75 mg/kg of NO_3^- -N in vegetated spoil and soil respectively. The rate of N mineralization in the vegetated spoil gradually decreased with duration of incubation but gradually increased in the undisturbed soil during the same period. Carbon dioxide evolution was similar in the vegetated spoil and soil samples throughout the incubation indicating that microbial activity in the two materials was comparable.

Reeder and Berg (1977a) used a pot experiment to measure N uptake by barley from the two materials described above. Plant N uptake was 21 mg/pot in both the vegetated spoil and the undisturbed soil. The authors suggested that the similarity in N mineralization rates and uptake values between the alfalfa-dominated, five year old spoil and the soil indicated that after 5 to 10 years of vegetative cover,

the ability of reclaimed geologic materials to cycle N may approach that of soil. This suggests that the active N component accumulates and reaches a steady state sooner than other parts of the system (McGill 1983).

The large amount of organic N measured in shale has prompted several studies on the capacity of this indigenous N to release mineral N (Power *et al.* 1974, Reeder and Berg 1977a,b). In an early study, Hall and Miller (1908) found that only 9.7 ppm NO_3^- was mineralized after a 14 month incubation of shale containing 1370 ppm total N. Aldag and Strzyszczyk (1980) measured levels of exchangeable NH_4^+ up to 110 ppm in coal spoils in Poland. Freshly exposed Paleocene shale contained 10 to 40 ppm NH_4^+ (Power *et al.* 1974). During a 22 week incubation this NH_4^+ was nitrified but no net increase in total mineral N content was observed. Similarly, Reeder and Berg (1977b) measured no net mineralization in a shale sample collected at a depth of 10 m. Fresh, nonvegetated spoil mineralized 5 ppm NO_3^- in the same study. The total amount of CO_2 evolved from the shale, 0.8 mg C/g, was similar to that from undisturbed soil. Less CO_2 -C evolved from the fresh spoil samples (0.4 mg/g), indicated less microbial activity in this material than in the shale. Plants grown in the shale or fresh spoil took up a total of 12 mg of mineral N from 1400 g material compared to almost twice that amount in vegetated spoil and soil samples (Reeder and Berg 1977a). It is evident from these studies that coal-bearing shales support microbial activity

and can mineralize N, but in quantities much smaller than vegetated spoil. This input of N may be important, however, to grasslands established on this material once fertilization has been terminated.

MICROBIAL ACTIVITY

The importance of soil macro- and microorganisms to organic matter decomposition and nutrient cycling has resulted in numerous studies of the biological activity of reclaimed mine spoil. Microbial parameters useful in reflecting the state of a reclaimed system have been reviewed by Cundell (1977), Hersman and Temple (1979) and Parkinson (1979). Soil respiration is frequently used as an index of total soil biological activity. Population counts of various physiological groups of bacteria and fungi, size of soil microbial biomass, enzyme activities and levels of adenosine triphosphate (ATP) are indirect indicators of microbial activity or soil organic matter content and quality.

Lawrey (1977a) found less diverse fungal populations and lower soil respiration in a bare strip-mined habitat than in a vegetated control habitat. This was attributed to low pH and a high metal content of the spoil. Hersman and Temple (1978) measured levels of ATP in reclaimed coal strip mines in Montana and observed that native range soils contained higher levels of ATP than the spoils. Microbial respiration, microbial biomass C, and ATP levels were all

significantly lower in an unreclaimed mined area (30 years old) than an undisturbed shortgrass prairie in southern Alberta (Visser *et al.* 1983). Bacterial numbers were much greater in the mine spoil than in the undisturbed soil and this was thought to be a result of domination by bacteria capable of maintaining a viable population in the low nutrient conditions. Fungal diversity was similar in the two areas under comparison but the population in the mine spoil was dominated by common air-borne fungi, all of which were early colonizers of recently dead and decomposing plant material. Microbial respiration and amylase activity increased with time on reclaimed mine spoil in West Virginia and after 20 years activity levels in the surface 10 cm approached those of native soils (Stroo and Jencks 1982). Phosphatase activity was low in these mined soils and urease activity followed no discernable pattern. Respiration, amylase and phosphatase activity were correlated with each other on the naturally revegetated spoils and all three indices were dependent on organic C and N levels. A three year old reclaimed area in New Mexico, seeded with native grasses and shrubs, had bacterial numbers and fungal diversity comparable to an adjacent, undisturbed soil but dehydrogenase activity was low (Fresquez and Lindemann 1982).

Accumulation of C and N in the surface layer of reclaimed mine spoil occurs at a rate determined by management practices, climate, spoil properties, and N_2 fixation. Measurement of microbial activity, litter

decomposition and N mineralization all indicate that N cycling processes occur to some extent in revegetated spoil.

III. DESCRIPTION OF THE STUDY AREA

The study area is situated on the property of Westar Mining Ltd. (formerly B.C. Coal) in the Rocky Mountains of southeastern British Columbia. This large surface coal mine has been in operation near the town of Sparwood since 1969. Approximately 160 ha of mine spoil have been reclaimed since 1974, and a further 2400 ha are proposed as the mine expands. The topography of the area is characterized by high ridges and peaks rising 1500 m above broad glacial valleys. The study sites are located at an elevation of 2000 m. The main stratigraphic units of the mountains are composed primarily of feldspathic sandstones, limestone and conglomerate with mudstone and fine to medium grained sandstone in the coal bearing formation (Dick 1978). The soils in the area consist mainly of Brunisols. Native grasslands occur on Sombric Brunisols or Humic Regosols and are characterized by thick Ah horizons (10 to 40 cm). Shallow Dystric Brunisols and Humo-ferric Podzols are found under forest vegetation (Ziemkiewicz 1979). Prior to mining, the vegetation of the high elevation reclaimed sites was subalpine forest with *Pinus contorta*, *Pinus albicaulis*, *Picea engelmannii* and *Abies lasiocarpa*. Understorey species include *Rhododendron albiflorum*, *Menziesia ferruginea*, *Vaccinium membranaceum* and *Vaccinium scoparium*. Grasslands also form a significant portion of the subalpine zone and tend to dominate on steep, southwest facing slopes.

The climate at the mine site is continental cold humid (Dick 1978). The climate recording station nearest the study site is operated by Westar Mining Ltd. and is located at the mine maintenance complex, 2 km northwest of the reclaimed sites and 200 m lower in elevation. Due to the difference in elevation between the reclaimed areas and the recording station, it is likely that temperatures at the research sites are lower, and precipitation is higher, than those recorded. Climatic observations recorded at this station during 1982 and 1983, and average temperature and precipitation measurements for 1976 to 1980 are tabulated in Appendix I. Snow frequently occurs in all months of the year and accounts for approximately 78% of the annual precipitation. Precipitation is heaviest from November to February and is relatively evenly distributed throughout the rest of the year. The growing seasons are short, cool, and moist with the ground snow-free for about five months of the year. The average frost-free period is 61 days (Dick 1978).

IV. THE MINING PROCESS AND SITE PREPARATION

The reclaimed mine spoil under study is the end product of open pit coal mining. During the mining operation, rock overlying the coal seams is shattered by blasting and removed by a truck and shovel operation. Topsoil is not conserved but becomes mixed with the waste rock during removal. Spoil from various pits within the minesite is deposited in a dump site in successive layers. When dumping has concluded in an area, dumps are resloped to an angle of 28° or less. The spoil surface is loosened using a heavy pipe stem harrow which creates a highly permeable surface layer about 10 cm deep. Underneath this layer, the spoil is often severely compacted by the weight of heavy machinery and may restrict downward movement of water and root penetration.

Once an area has been harrowed, seed and fertilizer are broadcast by helicopter. Fertilizer is applied annually after site establishment at a rate of 200 kg/ha of 13-16-10.

The predominant species in the seed mixture include *Festuca rubra*, *Phleum pratense*, *Dactylis glomerata*, *Poa canadensis*, *Poa pratensis*, *Alopecurus pratensis*, *Trifolium hybridum*, and *Medicago sativa*.

V. MATERIALS AND METHODS

A. FIELD EXPERIMENT

SITE SELECTION

Three reclaimed areas of different ages and an undisturbed native grassland were selected to represent the developmental sequence of a system following revegetation of a mined, subalpine site. The reclaimed sites were initially seeded and fertilized in 1980, 1977, and 1974 and at the time the study was initiated, were 2, 5, and 9 years old respectively. The 1980 and 1977 sites had been fertilized annually since site establishment but the 1974 site was fertilized annually only until 1978. This site was fertilized only once, in 1981, since that time. Trees along the perimeter of the native grassland were 150 years old and it is expected that the soil developed under the grassland is much older.

To reduce the variability between reclaimed sites due to climate and plant species composition, all plots were located on level ground with a southwest exposure, were within an elevational range of 120 m and supported a mixture of agronomic species. Species composition in the individual research plots was documented in August 1982 and the data are summarized in Appendix II.

The native grassland, situated adjacent to the 1974 site, differed from the reclaimed areas in several respects.

It was developed on a steeply sloping, 36°, deposit of sandstone colluvium which had physical characteristics similar to the waste rock of the reclaimed sites but had a smaller proportion of siltstone and mudstone. The percent coarse fragments was lower in the grassland soil than in the the reclaimed soils. The vegetation consisted entirely of native species including *Poa canbyi*, *Festuca idahoensis*, *Aster conspicuous*, *Lomatium dissectum*, *Lupinus seriseus*, and *Amelanchier alnifolia*.

EXPERIMENTAL DESIGN

Square plots, 2.5 m on edge, were established on each of the four sites in May 1982. Each plot consisted of a central area, 1.5 m on edge from which samples were taken, surrounded by a 0.5 m buffer zone (Figure 2). The central sampling zone was further divided into nine equal parts, each 0.5 m on edge. Four replicates of each plot were established at each site.

Trenches were dug to a depth of 15 cm around each plot to break roots traversing the fertilized and unfertilized areas. Trenches were lined with heavy plastic and filled with soil and rocks. All plots were covered with a wire mesh enclosure to prevent grazing. The mesh size was sufficiently large (2 cm) to prevent changes in the microclimate within the plot. The lid of the enclosure could be opened to permit sampling.

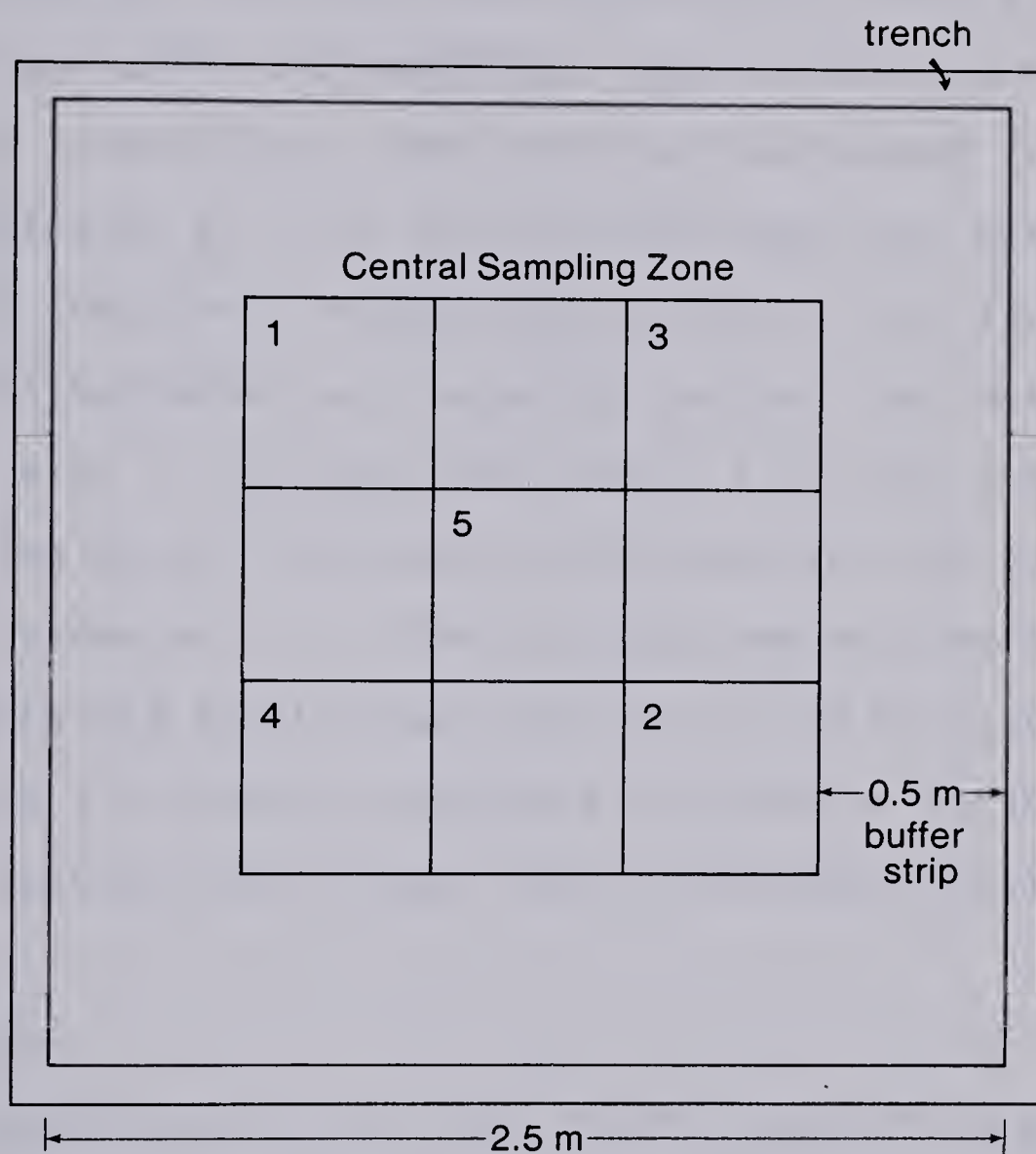


Fig. 2: Plot layout. Numbers indicate the order in which samples were removed from plots.

FERTILIZATION

The amount of fertilizer added to the research plots was similar to the rate used by Westar Mining for their annual maintenance fertilization (200 kg/ha of 13-16-10). On June 2, 1982, 2.6 g N/m² as (¹⁵NH₄)₂SO₄ (4.587 % excess) 3.2 g P/m² as K₂HPO₄ and CaH₄(PO₄)₂ and 2 g K/m² as K₂HPO₄ were applied to each plot. The fertilizer allocated to each plot was dissolved in 1.2 L of distilled water and sealed in a plastic container. In the field, the 1.2 L of fertilizer solution was added to a watering can and the container was rinsed with 1 L of distilled water. A further 2 L of distilled water were added to the watering can to make a final volume of 4.2 L. The solution was stirred thoroughly and sprinkled evenly over the entire 6.25 m² plot. Following the June 2 treatment, the plots received no further fertilization and no water other than precipitation.

SAMPLING

Samples were taken June 22-25, August 9-12 and September 14-17 in 1982, May 30-June 2 and August 9-12 in 1983 for a total of five sampling dates. One day was required to sample each site and four consecutive days of sampling were necessary to complete the sampling on all sites. Each sample was taken from one randomly selected part of the central sampling zone with the first four samples removed from the corners of the central zone and the final sample removed from the centre. This pattern,

described in Figure 2, was followed to minimize disruption of the soil and vegetation to be removed in subsequent samples. A metal square, 0.5 m on edge was used to define the sampling area. Samples were collected outside the fertilized plots for the measurement of natural abundance ^{15}N in June 1983 (Appendix III).

Shoots

All living, nonlegume plant material was referred to as the shoot component. When legumes, such as alfalfa or clover occurred, they were collected separately. All living material was clipped at ground level and placed in a paper bag.

Litter

All dead plant material, including that still standing and attached to the plant, and that lying on the soil surface, was included in the litter component. Litter was collected from within the sampling square as described above, and placed in a paper bag. When conditions were too wet to permit sorting of litter from shoots, all plant material was placed unsorted in a paper bag, dried overnight and sorted in the laboratory.

Crowns

The definition of crowns as an easily distinguishable physical entity is difficult (Sims and Singh 1978). In this study, the base of the plant from which the stems and roots originate was termed the crown. Once the shoots and litter

had been removed, the soil surface was loosened and all plant crowns in one quarter of the square removed. Attached roots were clipped close to the base of the crown, adhering soil or stones were removed and the crowns were placed in paper bags.

All shoot, litter and crown samples were dried for 24 h at 70°C, weighed and yields reported in Appendix IV. Samples were ground to approximately 20 mesh using a blender, mixed thoroughly and a subsample taken. This was further ground to 40 mesh using a Wiley Mill.

Soil

Soil material was removed from the same quarter of the sampling square as the crowns and collected to a depth of 10 cm. In most plots compaction and large rocks necessitated the use of a rock hammer to loosen the material. All material, including rocks, soil and roots, was collected, placed in plastic bags and air-dried in the laboratory. After seiving and root removal, described below, the subsamples of soil were ground to 100 mesh using a Siebtechnik laboratory disc mill (model TS100A). Future references to soil samples in this work refer to the material from which rock fragments larger than 2 mm and all roots have been removed.

Roots

After air drying, the soil material was seived and vacuumed to extract roots in a manner similar to that

described by Clark (1977), with large roots picked from the soil sample by hand and the remainder separated from the soil by air suction as follows: A portion of the soil sample was placed on a metal tray and moved back and forth over a hand-held vibrating tool which was securely clamped to a stand. The vibration caused root and litter fragments to move to the surface of the sample, where they could be picked off by suction. A piece of fine nylon gauze was placed over the end of a vacuum cleaner nozzle which was held parallel to, and 1 to 2 cm above, the vibrating surface. Debris collected on the gauze surface was removed at regular intervals to prevent loss of suction. The soil material was vacuumed until no further debris accumulated on the gauze. The entire soil sample was treated in this manner.

Coarse root fragments were defined as those longer than 1 cm and wider than 1 mm; fine root fragments were the remainder. This boundary was approximate and subjective and was based on the premise that coarse roots were generally sieved out with the coarse fragments and could be picked by hand, while fine roots had to be vacuumed from the less than 2 mm fraction. The fine root component became thoroughly mixed with litter fragments during separation from the soil and required that a subsample be taken to determine the proportion of fine roots in the mixture. The weights of the coarse and fine roots were determined and yield calculated per m^2 to a depth of 10 cm. All coarse root fragments were

ground to 40 mesh using a Wiley Mill. Fine root size was too small to permit grinding and analyses were performed on unground samples.

B. MICROBIAL BIOMASS ESTIMATION

SAMPLE PREPARATION

Soil samples for biomass measurements were collected on each of the five sampling dates in 1982 and 1983. Samples of about 1 kg were removed from within the sampling square, adjacent to the soil samples removed for root extraction and total nitrogen analysis. One sample was taken from each plot with a total of four per site. The soil was collected in plastic bags and taken to the lab within a few hours. Samples were passed through a 10 mesh seive to remove large roots and coarse fragments, and stored overnight at 15°C in airtight containers. A subsample was taken at that time and dried at 105°C to determine field moisture content. The following day, samples were brought to 55 % of water holding capacity and duplicate samples (25 g O.D.B.) were weighed into 100 mL beakers.

CHLOROFORM FUMIGATION

Moist samples were fumigated with ethanol-free chloroform for 18 hours as described by Jenkinson and Powlson (1976a). Following evacuation of the chloroform vapours, samples were incubated in sealed 2 L canning jars

at 25°C for 11 days.

CO₂ EVOLUTION

The CO₂ evolved from the reclaimed samples was trapped in 0.25 M NaOH (20 mL). The native grassland samples required 30 mL of 0.25 M NaOH. The amount of CO₂ collected during the 11 day incubation was measured by back titration with standardized 0.1 M HCl, after precipitating the carbonate with 2 mL of 4 M BaCl₂ (Stotzky 1965).

NITROGEN MINERALIZATION

At the end of the incubation period, soil samples were extracted with 125 mL of 4 M KCl. Phenyl mercuric acetate (5 ug/mL) was added to the KCl extracting solution to reduce the possibility of microbial growth (Douglas and Bremner 1970). Samples were extracted at the Westar environmental laboratory and extracts were kept frozen until analyzed at the University of Alberta several weeks later.

Mineral nitrogen ($\text{NH}_4^+ + \text{NO}_3^-$) was determined by steam distillation (Bremner 1965b). Samples containing less than 0.4 mg of N per sample were spiked with 1 mg of natural abundance $(\text{NH}_4)_2\text{SO}_4$. Samples were acidified, dried and abundance ^{15}N measured as described in Part E.

C. NITROGEN MINERALIZATION POTENTIAL

Soil samples collected from the fertilized field plots in August 1982 were used to determine potentially mineralizable nitrogen using a modified leaching-incubation method of Legg *et al.* (1971). Glass microfibre filter paper was placed on the bottom of plastic 6.4 cm diameter Buchner funnels and 40 g of air dry soil was added to each. The soil surface was covered with a layer of glass wool and the sample moistened to 55 % of water holding capacity. Each funnel was leached initially with 100 mL of 0.01 M CaCl_2 followed by 25 mL of a minus-N nutrient solution (Stanford and Smith 1972). Excess moisture was removed by 0.6 bars suction and the funnels of soil were incubated in sealed plastic bags at 30°C. Samples were weighed periodically to determine water loss and remoistened as required. After intervals of 2, 2, 4, 4, 6 and 5 weeks (23 weeks total) samples were leached as described above. Leachates were analysed for NO_3^- and NH_4^+ using steam distillation (Bremner 1965b).

D. NITROGEN FIXATION ASSOCIATED WITH GRASS ROOTS

SURVEY TO DETERMINE PRESENCE OR ABSENCE OF NITROGEN FIXERS

Root samples were collected from the four research sites in the vicinity of the established plots in August 1982 and June and August 1983. Several grass species were sampled. Healthy looking plants were removed from the soil

with their roots intact, placed in plastic bags and kept in a cooler until transported to the laboratory. Excess soil was gently shaken from the roots and 1 to 2 cm pieces of growing root tips were placed in vials of N-free media (Appendix V). Samples were incubated for 10 days at room temperature and then stored in a refrigerator at 5°C until they could be transported to the University of Alberta for further analysis. At the University, an aliquot (1-2 mL) of each culture was transferred to 20 mL of fresh N-free media in 40 mL serum bottles, was injected with 5 mL of purified acetylene and left for 24 hours at room temperature. Gas samples (1 mL) were analysed for ethylene on a flame ionization gas chromatograph (Hewlett Packard 5790A Series) using a Porapak N column.

NITROGEN FIXATION UNDER FIELD CONDITIONS

Once the presence of nitrogen fixers on the study sites was established an attempt was made to estimate their activity under field conditions. The method used was adapted from Rice (1980) as follows: healthy specimens of several grass species were removed intact from the soil; soil was shaken from the roots and individual plants were placed in a 1 L glass Mason jar which was capped with a lid fitted with a serum stopper. Purified acetylene (100 mL) was injected into the jars through the serum stopper using a disposable syringe. The jars were then placed in the hole from which the plant had been removed and covered with soil. Samples

were incubated in the field for 24 hours, transported to the laboratory for immediate analysis of ethylene as in D. Several alsike clover plants were included in the study to ensure that the method worked properly.

E. ANALYTICAL METHODS

PHYSICAL ANALYSIS

Bulk Density

In the field experiment, once the soil and rock material was removed from the plot, the resulting hole was lined with a plastic bag and filled with water to determine sample volume. The sample was air-dried, weighed and its bulk density calculated (Appendix VI). The bulk density of the soil and rock material was determined at each of the 5 sampling dates for a total of 20 measurements per site.

Coarse Fragments

In 1982, all air-dry samples were passed through a 10 mesh seive. Fragments larger than 2 mm were termed coarse and the percentage of coarse fragments by weight in the soil material was calculated. In 1983, samples were seived through a 5 mesh seive, in addition to the 10 mesh seive, to measure the proportion of larger fragments (Appendix VI). Soil mass (g/m^2 to 10 cm) was calculated by subtracting the weight of coarse fragments in the sample from the total

weight of soil material.

Particle Size Analysis

Particle size distribution in two soil samples from each of the four sites was measured using the hydrometer method of Day (1965). Organic matter was removed with hydrogen peroxide prior to analysis. Particle size data are summarized in Appendix VI.

Water Holding Capacity

Soil samples were placed in funnels lined with filter paper and saturated with distilled water. Samples drained rapidly but were left for 6 hours before a subsample was taken and moisture content determined as an estimate of water holding capacity.

CHEMICAL ANALYSIS

Total Nitrogen

Soil samples were pretreated with KMnO_4 and H_2SO_4 to oxidize nitrite to nitrate, and then with reduced iron to reduce nitrate to ammonium (Bremner 1965a). The micro-Kjeldhal method with steam distillation was used to determine total nitrogen in all plant and soil samples (Bremner 1965b). Samples were digested for 1.5 hours at 220°C and 4 hours at 360°C in 250 mL tubes on a Technicon BD-20 heating unit. They were cooled, diluted with 20 mL distilled water and distilled with steam directly from the

digestion tubes on an appropriately modified microdistillation unit. The distillate was collected in 10 mL of 2% boric acid and titrated to a pH of 4.8 with 0.005 M H_2SO_4 using a Mettler DL 40 RC Memotitrator.

Abundance of ^{15}N

Following titration, samples with an abundance greater than 1.0% were spiked with 1 mg of natural abundance $(\text{NH}_4)_2\text{SO}_4$. The distillate was acidified with 0.3 mL of 0.5 M H_2SO_4 and taken to dryness on an 80°C sandbath. The quantity of 0.5 M H_2SO_4 used for acidification was reduced to 0.1 mL part way through the study. The salts were redissolved, transferred to disposable culture tubes and dried at 60°C in the oven. Ammonium was oxidized to N_2 using LiOBr according to the method of Porter and O'Deen (1977). Isotope ratio analysis was performed on a Micromass 602C double collector mass spectrometer using atmospheric N_2 as a reference. Standards were run daily to ensure analytical precision. Standard error of ^{15}N abundance of bottle $(\text{NH}_4)_2\text{SO}_4$ standards, run over a period of several weeks was 0.017% of the mean (0.3658 %abundance ^{15}N).

Cation Exchange Capacity

The total exchange capacity of four soil samples from each site was measured by the sodium acetate method (McKeague 1978). The concentration of Na was determined on a Perkin Elmer 303 atomic absorption spectrophotometer. Cation exchange capacity data are recorded in Appendix VI.

F. STATISTICAL ANALYSIS

Samples were collected from four sites with four replicates from each site. During statistical analysis, replicates were treated as simple random samples. All parameters were subjected to a one way analysis of variance across dates and across sites. The significance of differences between means was tested using the Student-Newman-Keuls test.

VI. RESULTS AND DISCUSSION

A. DISTRIBUTION OF NITROGEN AMONG PLANT COMPONENTS

Quantities of N in the shoot, crown, root and surface litter components were compared between sites to assess changes in system development with site age. The proportion of total N in each of the components was calculated to determine which parts of the system acted as major sinks for N. Between-site differences in the pattern of N distribution were used to characterize changes in system properties which occurred with site age.

Quantities of labelled N in plant and soil components were compared between study sites to assess the fate of added N in reclaimed sites of different ages. Net gains and losses of labelled N by individual components provided an estimate of component turnover rate. Loss of labelled N from the system and the efficiency with which plants utilized applied fertilizer were also measured.

RESULTS: TOTAL NITROGEN

1980 Site

Most of the N in plant components of the 1980 site (70-80%) was distributed evenly between the crown and root components on all sampling dates (Figure 3). The high proportion of N in the shoots in September 1982 was followed by an increase in the proportion of N in the litter in 1983.

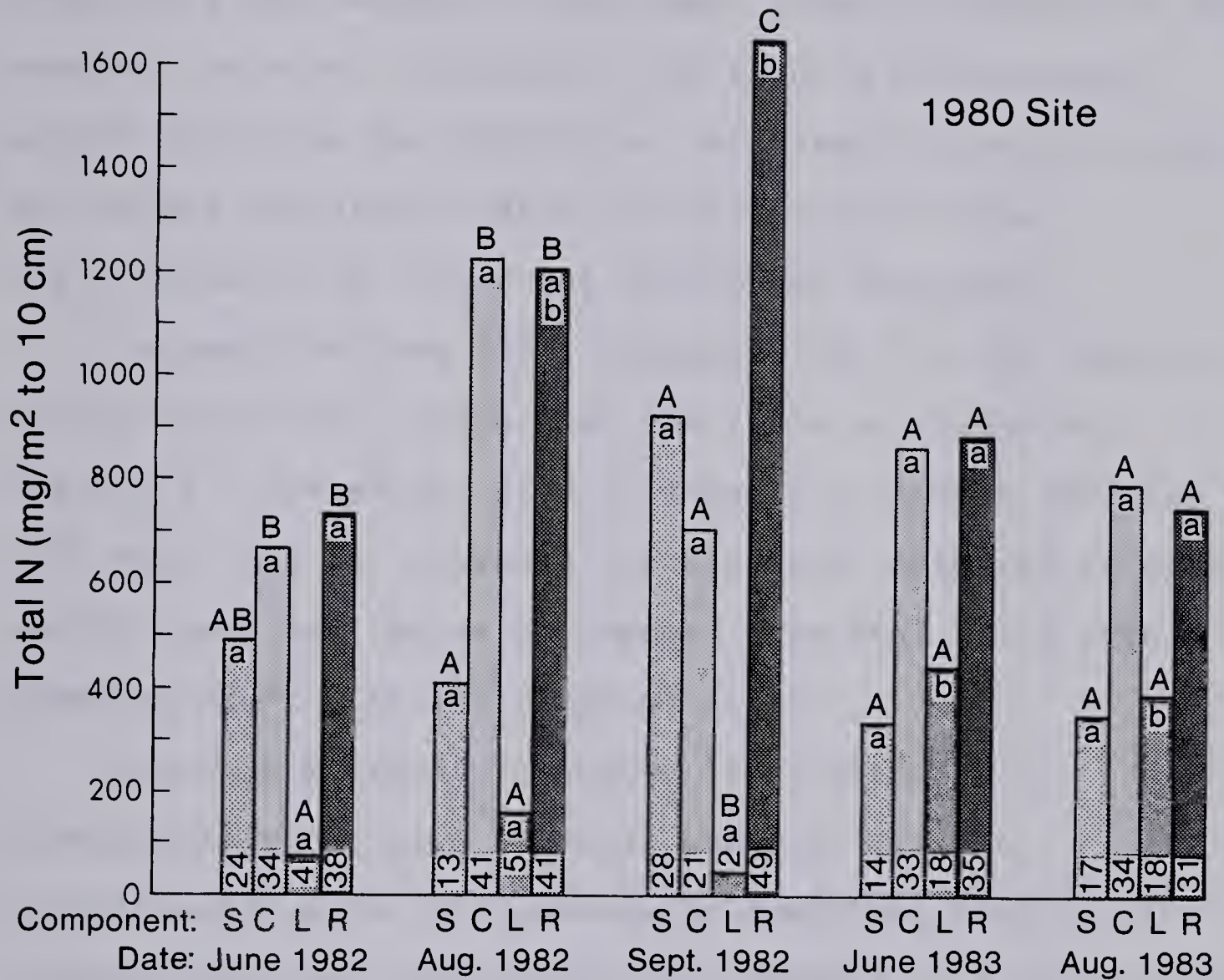


Fig. 3: Distribution of N among shoots(S), crowns(C), litter(L) and roots(R) at the 1980 site. Different lower case letters indicate significant differences ($p < 0.05$) in the amount of N in any one component over time. Different upper case letters indicate significant differences ($p < 0.05$) in the amount of N among components on any date. Numbers at the column base are the % of the total N in each component at any site and on any date.

Legume growth was sparse in the reclaimed area when the study sites were selected in the spring of 1982. For this reason, legumes growing within the plots were collected separately but because of the small biomass measured in most samples, were not included in the shoot N calculations. Legume growth on the 1980 site, increased during the study period and the significance of this growth to the distribution of N within the system was evaluated.

Legume N in June 1982, composed 14% of total shoot N but by August this proportion had risen to almost 40% (Figure 4). The amount of N in legumes in August 1983 was 1500 mg/m² and far exceeded the 350 mg/m² measured in grass shoots. Labelled legume N composed less than 5% of total labelled shoot N at all sampling dates.

Legume N measured in August 1982, although a substantial proportion of total shoot N, did not significantly alter the previously described distribution of N among the plant components. High amounts of N in roots and crowns overshadowed the combined grass and legume shoot N at this date. Total shoot N in August 1983, however, was twice that of any of the other components on the 1980 site and higher than total shoot N on any of the other reclaimed sites. Such an increase in shoot N suggested that considerable inputs of N to the 1980 site occurred through N₂ fixation. N₂ fixation by legumes was also indicated by the low %excess of legume N relative to the higher %excess of grass N.

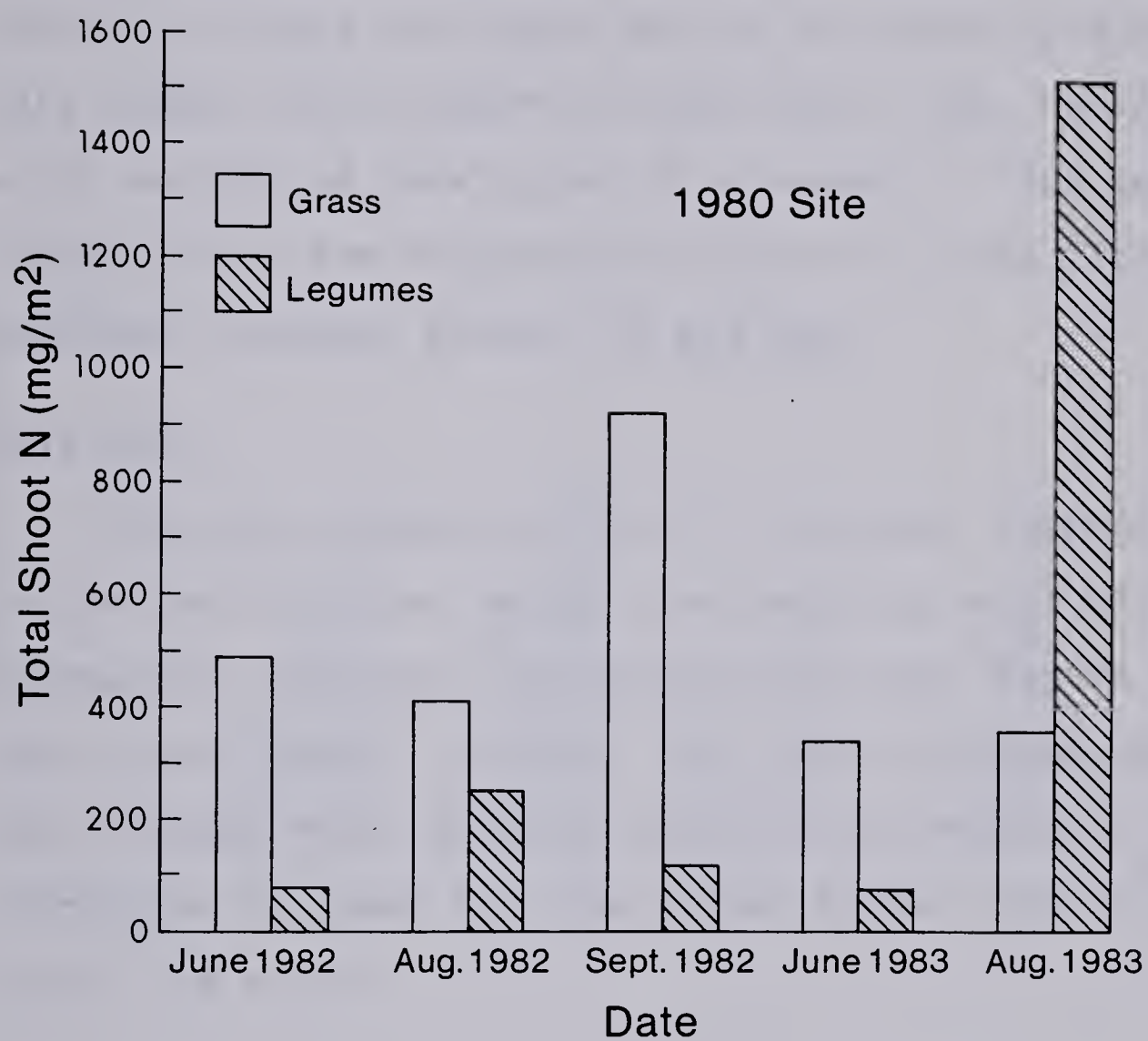


Fig. 4: Distribution of N among grass and legume shoots at the 1980 site.

1977 Site

The relative distribution of N among the plant components on the 1977 site changed during the study (Figure 5). The roots contained the major portion of plant N (40-60%) in June and August but as the roots died back in late summer, this proportion declined to 35%. Crown N peaked at 30 and 23% of total plant N in August of 1982 and 1983, respectively. The proportion of plant N in the litter component remained between 18 and 25%.

1974 Site

The major proportion of N in the plant components was in the roots at four out of five sampling dates although substantial seasonal fluctuations occurred (Figure 6). As root growth peaked in August 1982, root N accounted for over 50% of plant N but declined later in the season to 34%. The proportion of plant N in the litter ranged from 22 to 33% during the study.

Grassland

Roots contained between 40 and 55% of the total N in plant components at all dates except in June 1982 (Figure 7). Litter accounted for 30-45% of the total plant N at four out of five sampling dates. The crown and shoot components contained relatively insignificant proportions of the total N throughout the study.

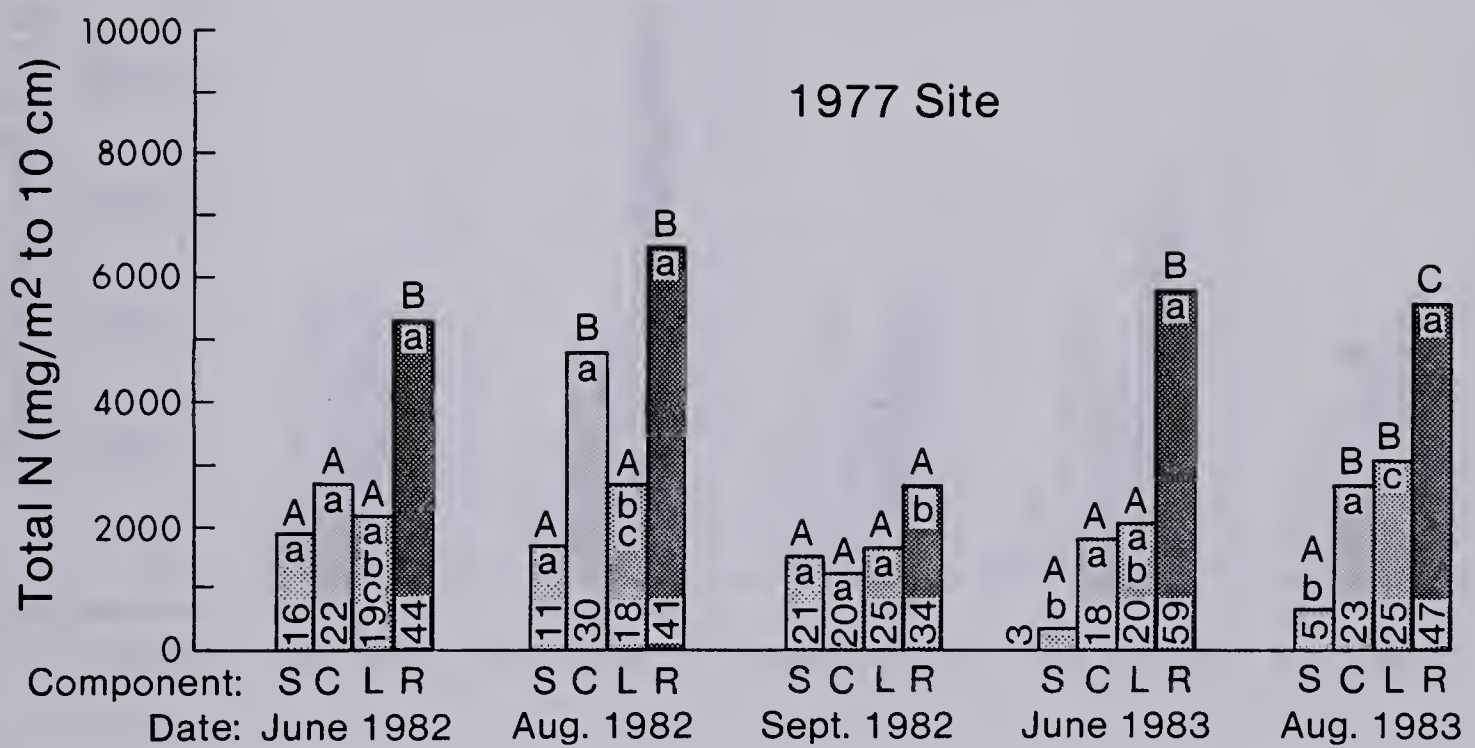


Fig. 5: Distribution of N among shoots(S), crowns(C), litter(L) and roots(R) at the 1977 site. See Figure 3 for a complete description.

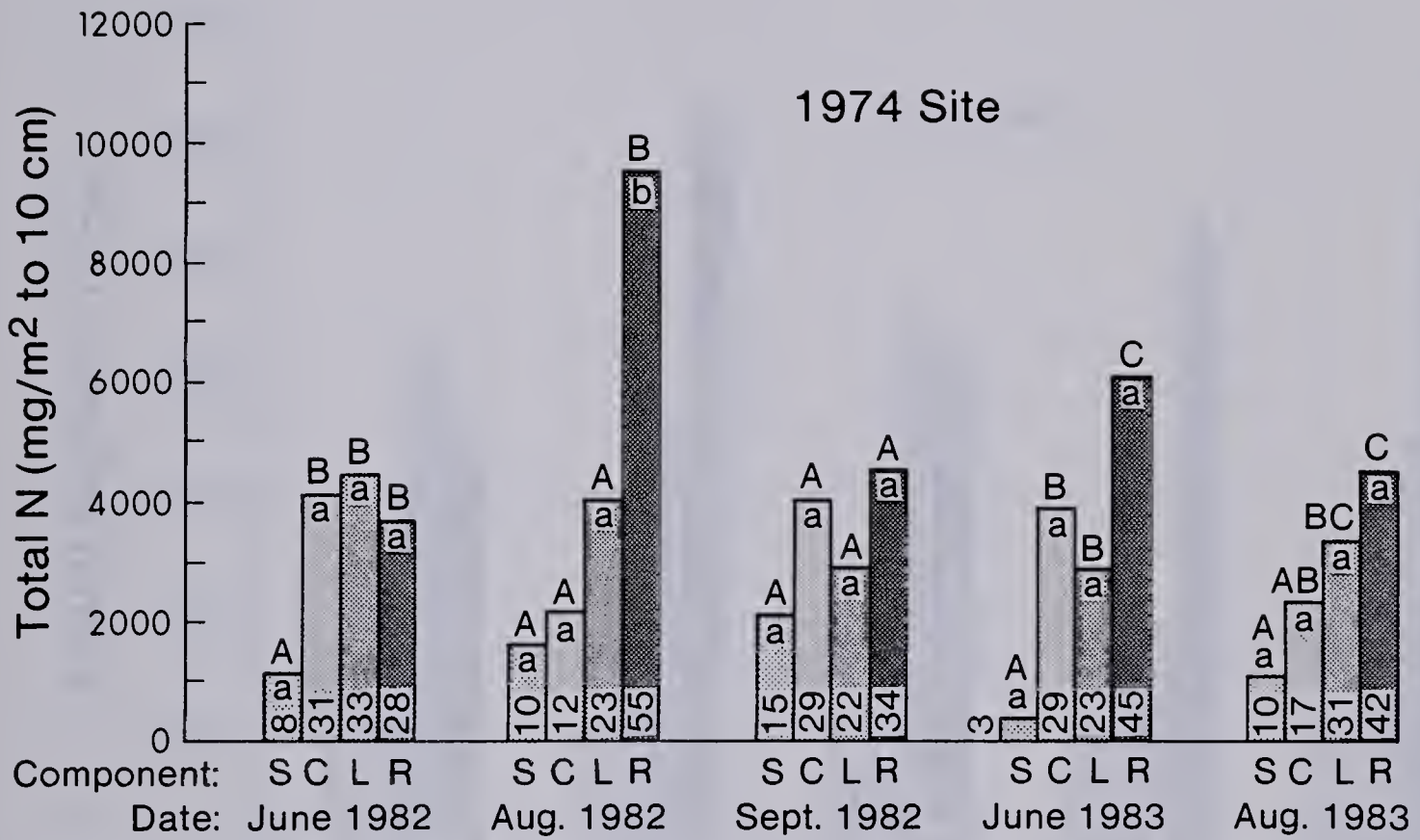


Fig. 6: Distribution of N among shoots(S), crowns(C), litter(L) and roots(R) at the 1974 site. See Figure 3 for a complete description.

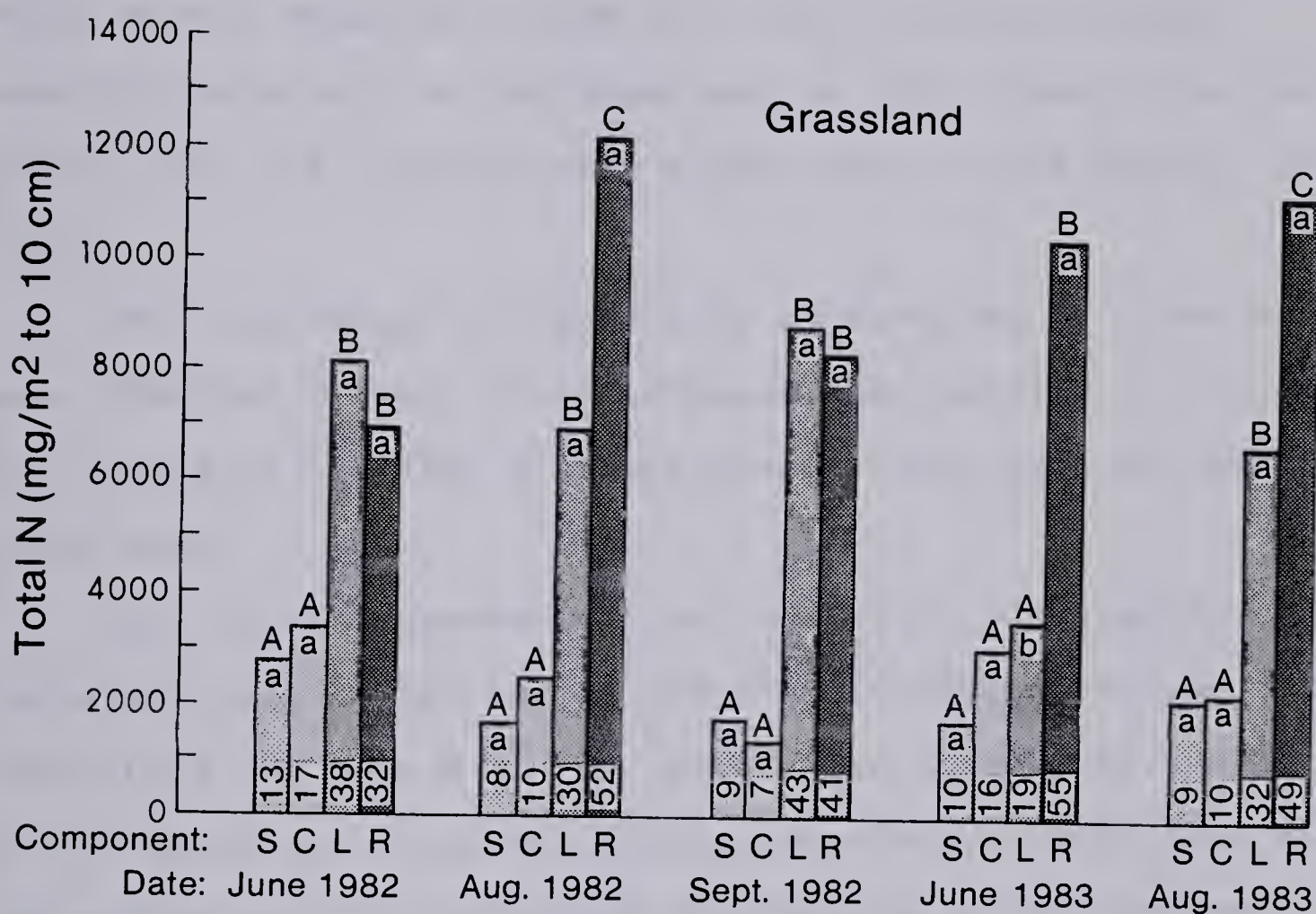


Fig. 7: Distribution of N among shoots(S), crowns(C), litter(L) and roots(R) at the native grassland. See Figure 3 for a complete description.

Between-site Differences

The amount of N in the shoots on the 1980 site was low relative to the other sites in the first few months of the 1982 growing season although by September 1982, this difference had disappeared (Table 5). Similar quantities of shoot N were measured on the 1977 and 1974 sites at all sampling dates and on the grassland in 1982. Shoot N on the latter site was significantly higher than on the others, in 1983.

Few statistically significant differences in crown N were observed between sites although the quantity of N in the crowns on the 1980 site was always lower than on any other site.

The litter component on the 1980 site consistently contained less N than any of the other study sites. The 1974 site contained more N in the litter than did the 1977 site at all dates but these differences were only significant on the spring sampling dates and disappeared as the growing season progressed. Litter on the native grassland contained a larger quantity of N than did litter on any of the reclaimed sites.

The quantity of N in the roots was significantly lower on the 1980 site than on the other sites at all sampling dates except September 1982. Root N did not differ between the 1977 and 1974 sites but was generally higher on the grassland than on any of the reclaimed sites.

Table 5. Quantity of N in plant components on four subalpine sites at Westar Mining Ltd. in southeastern B.C. (mg/m² to 10 cm)

| DATE | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
|---------------|-------------------|--------------|--------------|---------------|
| SHOOTS | | | | |
| June 1982 | 491a ¹ | 1960bc | 1160ab | 2820c |
| Aug. | 404a | 1730b | 1730b | 1710b |
| Sept. | 934a | 1500a | 2100a | 1750a |
| June 1983 | 343a | 307a | 435a | 1720b |
| Aug. | 353a | 607ab | 1120b | 2170c |
| CROWNS | | | | |
| June 1982 | 673a | 2700ab | 4180ab | 3410b |
| Aug. | 1240a | 4840b | 2160ab | 2500ab |
| Sept. | 719a | 1470a | 4030a | 1430a |
| June 1983 | 876a | 1840ab | 3950b | 3170ab |
| Aug. | 806a | 2720a | 1890a | 2260a |
| LITTER | | | | |
| June 1982 | 74a | 2240b | 4500c | 8200d |
| Aug. | 161a | 2750b | 4020b | 6980c |
| Sept. | 48a | 1760ab | 2930b | 8850c |
| June 1983 | 447a | 2020b | 2940c | 3510c |
| Aug. | 396a | 3000b | 3420b | 6830c |
| ROOTS | | | | |
| June 1982 | 737a | 5310bc | 3780b | 7090c |
| Aug. | 1230a | 6500b | 9520bc | 12260c |
| Sept. | 1660a | 2720a | 4580a | 8270b |
| June 1983 | 892a | 5910b | 6150b | 10740c |
| Aug. | 753a | 5610b | 4680b | 11300c |

1. Different letters indicate significant differences ($p \leq 0.05$) in component N between sites on any one date.

While the distribution of N among the plant components differed between sites, the roots generally contained a major proportion of the total plant N. The dominance of the crown component was evident only on the 1980 site. With few exceptions, the crowns of the other sites contained a small proportion of total plant N. Litter N generally accounted for a larger proportion of plant N in the grassland system than in any of the reclaimed systems.

RESULTS: LABELLED NITROGEN

Total recovery of labelled N

The proportion of fertilizer recovered in the plant and soil components fluctuated during the study period and variability within sites was high, yielding few statistical differences between dates (Table 6). Total recovery of added N in the three reclaimed systems ranged from 64 to 98%. Recovery was high in the native grassland throughout the study period with the exception of a substantial decrease in September 1982. On average, the reclaimed sites lost 20 to 25% of the added N within one year of application but no loss from the native grassland was apparent.

Labelled N recovered in plants

Peak recovery of added N in living plants (shoots+crowns+roots) coincided with peak recovery in the entire system on all sites in 1982 (Table 7). The 1980 site

Table 6. Percent of added fertilizer recovered in soil and plant components on four subalpine sites at Westar Mining Ltd.

| DATE | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
|-----------------------------|---------------------|--------------|--------------|---------------|
| June 1982 | 66.9ax ¹ | 67.2ax | 63.3ax | 118 ay |
| Aug. | 64.7ax | 97.6ax | 108 bx | 92.0ax |
| Sept. | 96.3ax | 68.1ax | 54.4ax | 55.8bx |
| June 1983 | 64.0ax | 72.7ax | 90.4abx | 125 ay |
| Aug. | 81.6ax | 80.5ax | 76.8abx | 103 ax |
| Average Recovery | 74.7x | 77.2x | 78.6x | 99.0x |

Table 7. Percent of added N fertilizer recovered in plants on four subalpine sites at Westar Mining Ltd.

| DATE | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
|-----------------------------|---------------------|--------------|--------------|---------------|
| June 1982 | 19.1ax ¹ | 49.1ay | 40.6ay | 45.6abcy |
| Aug. | 31.2ax | 68.5by | 76.0by | 50.5abxy |
| Sept. | 47.6ax | 41.6ax | 32.6bx | 25.3cx |
| June 1983 | 28.0ax | 38.8axy | 43.2axy | 56.0ay |
| Aug. | 23.0ax | 36.0ax | 34.9ax | 31.2bcx |
| Average Recovery | 29.8x | 46.8y | 45.5y | 41.7y |

1. Different letters (a,b,c) indicate significant differences ($p < 0.05$) between sampling dates in any one site.
 Different letters (x,y,z) indicate significant differences ($p = 0.05$) between study sites on any one date.

had the lowest recovery at all dates except September 1982 and % recovery in plants at this site never exceeded 48%. Maximum fertilizer recovery occurred on the 1977 and 1974 sites in August 1982 when plants contained 69 and 76% of the applied N respectively. By September, recovery decreased by 25-30% on the two older reclaimed sites and the native grassland. A continual increase was observed on the 1980 site throughout the growing season.

The following spring, a substantial amount of the fertilizer was recovered in the plants, particularly at the three oldest sites. This recovery was maintained into August on the 1977 site but a noticeable decline occurred in the 1974 and grassland sites.

1980 Site

Seasonal changes in the amount of labelled N in each component were similar to those described for total N (Figure 8). No significant change occurred in labelled soil N during the study period although fluctuations were evident.

Over 49% of the total labelled N on the 1980 site was in the soil at all sampling dates. Labelled N present in plant materials was evenly distributed between shoot, crown and root components in 1982 and between all plant components in 1983.

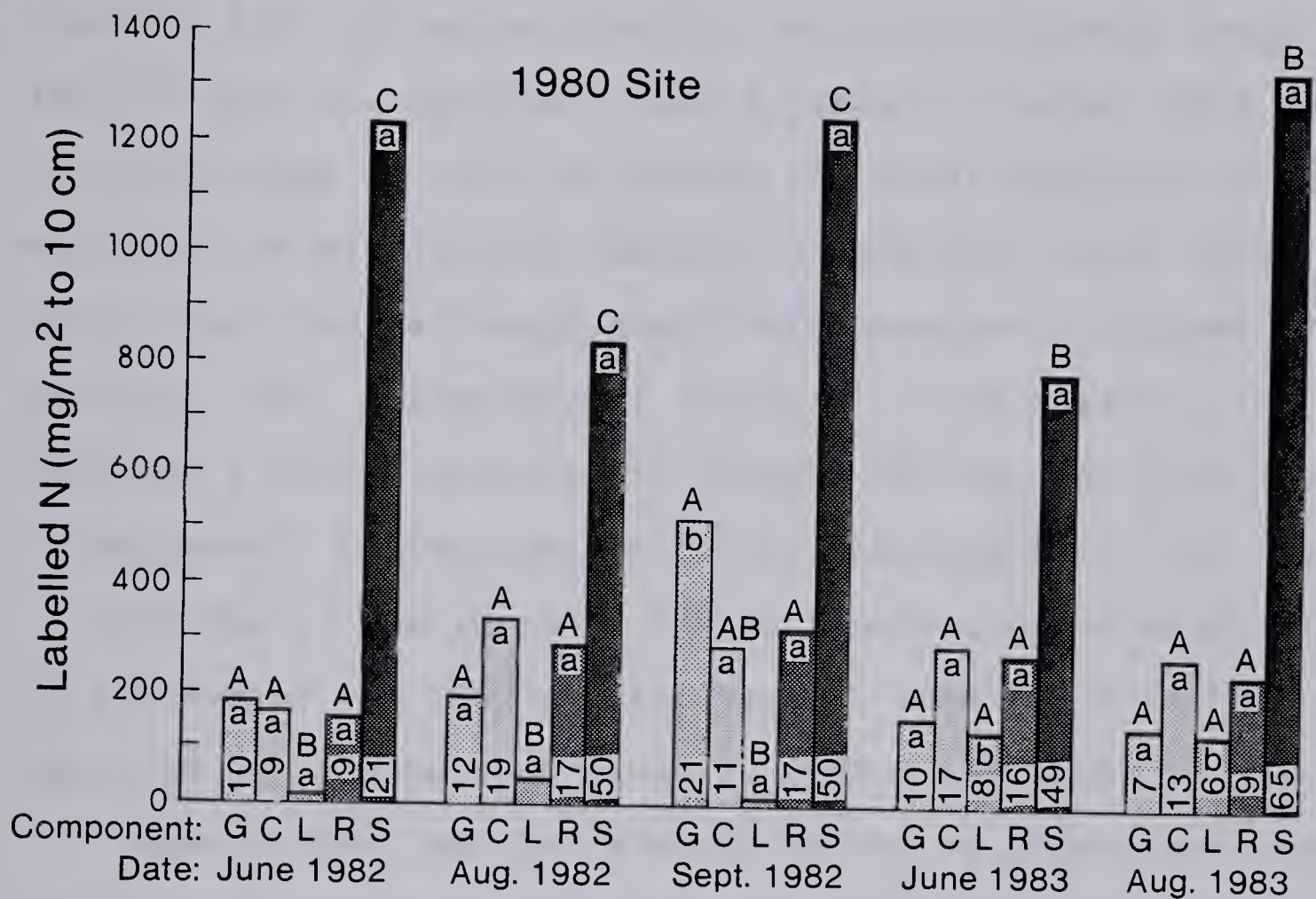


Fig. 8: Distribution of labelled N among shoots(G), crowns(C), litter(L), roots(R) and soil(S) at the 1980 site. Different lower case letters indicate significant differences ($p \leq 0.05$) in amount of labelled N in any component over time. Different upper case letters indicate significant differences ($p \leq 0.05$) in amount of labelled N among components on any date. Numbers at the column base are the % of total N in each component at any site and on any date.

1977 Site

The quantity of labelled N in shoots of the 1977 site remained high throughout 1982 but was significantly lower in 1983 (Figure 9). Labelled crown N peaked in August 1982 but no differences in crown N between the other sampling dates occurred. No significant changes in labelled root N occurred during the study although peaks were observed in August 1982 and June 1983. A significant increase in the quantity of labelled litter N occurred in August 1982 but declined again in September. Litter contained more labelled N in 1983 than in 1982 due to the input of highly labelled plant material at the end of the 1982 growing season. Labelled N in the soil did not change significantly during the study.

Most of the labelled N was utilized by plants and less than 30% of the total labelled N in the system was present in soil at any sampling date. A large proportion of the labelled N in June 1982 was in actively growing shoots but by August this had declined as the proportion of labelled N increased in the crowns. In September the reverse trend occurred; the proportion of labelled N in soil increased by over 10% and remained at this level throughout 1983. Shoots contained less, and litter more, of the labelled N in the system in 1983 than in 1982.

1974 Site

In 1982, the quantity of labelled shoot N did not vary significantly on the 1974 site; but during 1983 it declined

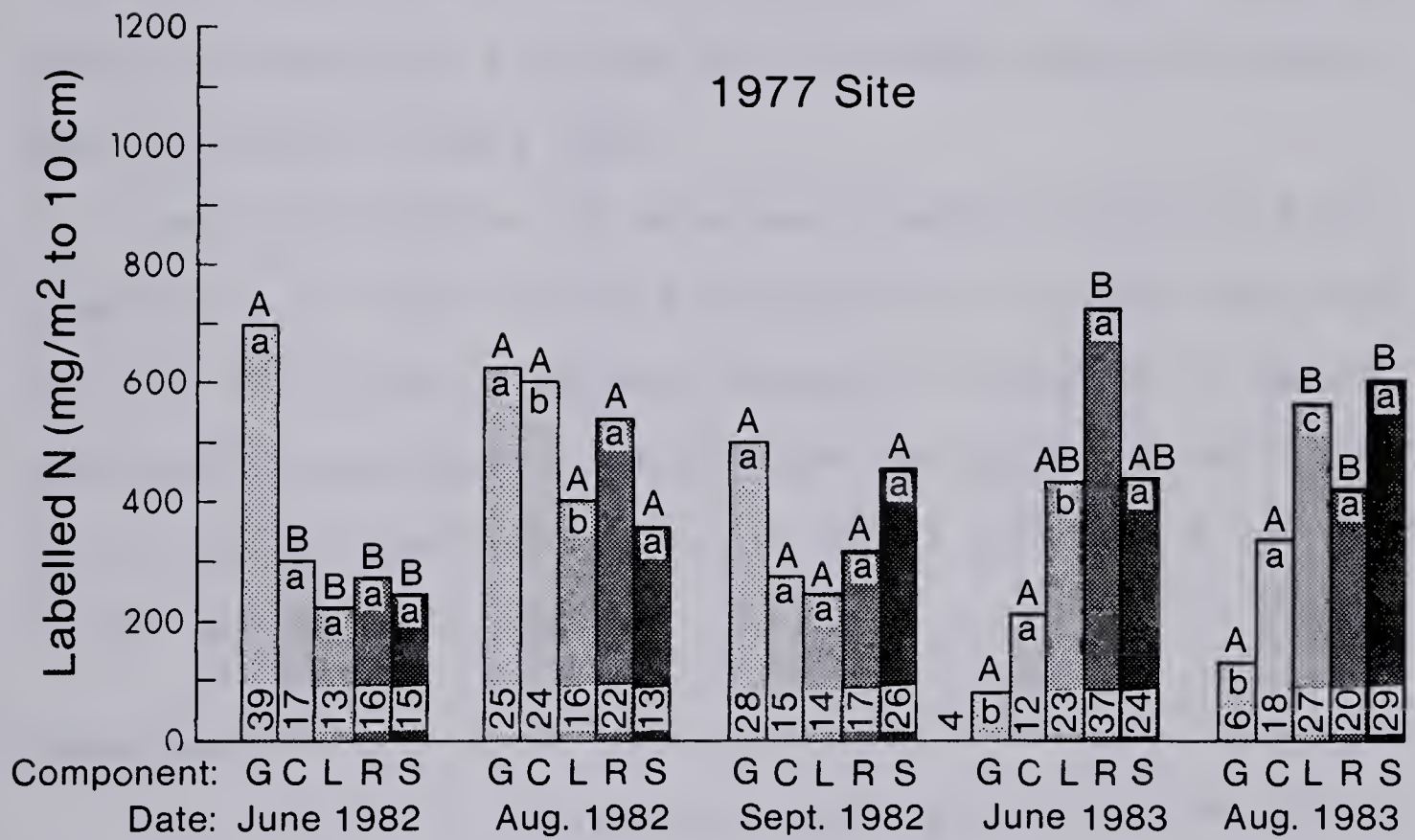


Fig. 9: Distribution of labelled N among shoots(G), crowns(C), litter(L), roots(R) and soil(S) at the 1977 site. See Figure 8 for a complete description.

by 70% (Figure 10). No seasonal change was measured in labelled crown N. The amount of labelled N in the litter reached a minimum in September 1982 but increased significantly in 1983. Seasonal changes in labelled root N were similar to changes in total N with a maximum occurring in August 1982. There was no significant difference in the amount of labelled N in the soil between dates although a peak occurred in June 1983.

The distribution of labelled N among plant and soil components at the 1974 site was similar to that described for the 1977 site. A notable exception occurred in August 1982 when large quantities of labelled N in the roots of the 1974 site represented 41% of the total labelled N compared to 22% on the 1977 site.

Grassland

The amount of labelled N in the shoots of the native grassland declined steadily throughout the sampling period although the only statistically significant change occurred between June and August 1982 (Figure 11). Labelled crown, litter and root N did not change in a statistically significant manner during the study but noticeable peaks in labelled root N occurred in June 1982 and June 1983. Labelled soil N declined significantly during 1982 increasing again in 1983.

Together, soil and root components of the grassland generally contained most of the total labelled N in the system. Approximately 50% of the total labelled N in June

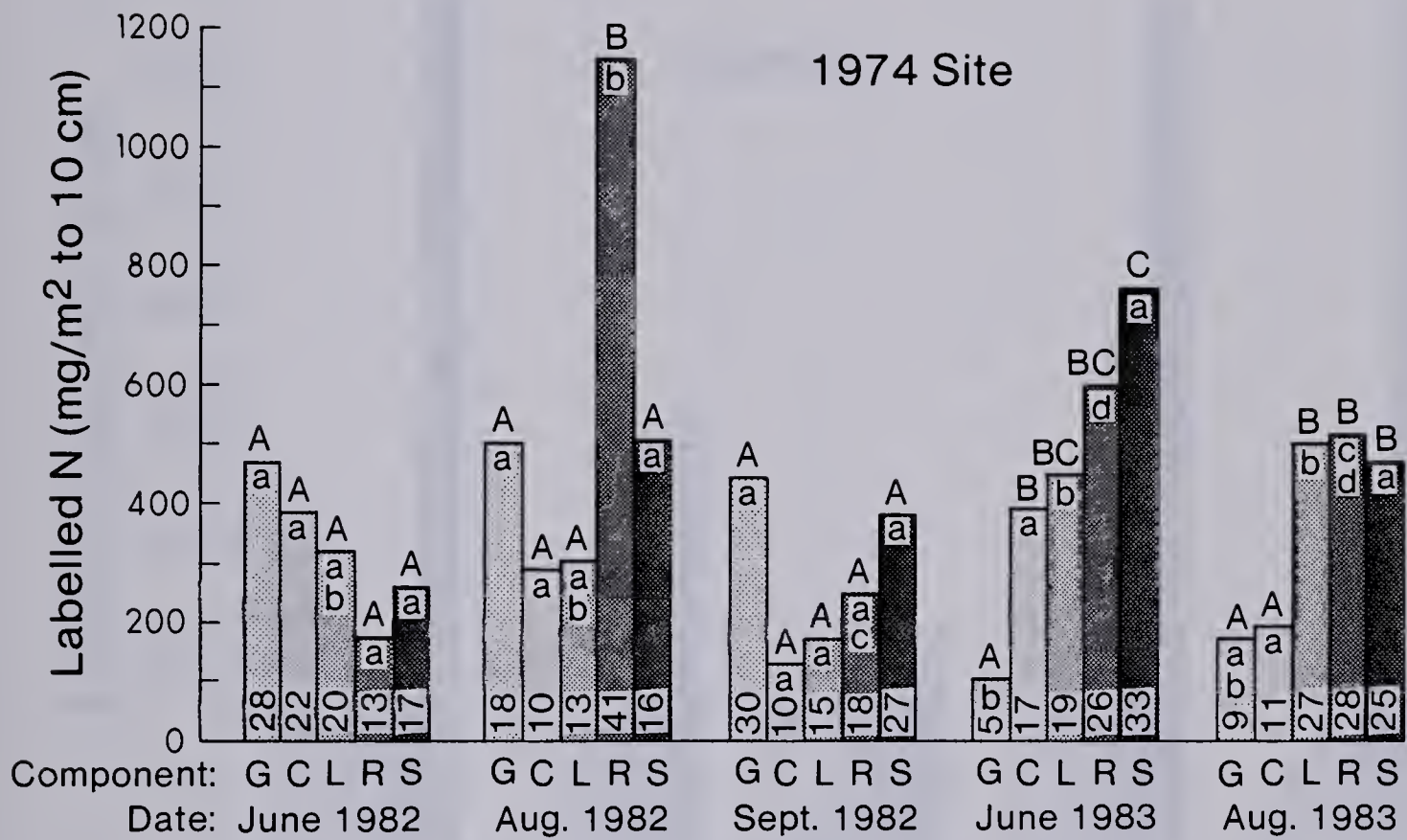


Fig. 10: Distribution of labelled N among shoots(G), crowns(C), litter(L), roots(R) and soil(S) at the 1974 site. See Figure 8 for a complete description.

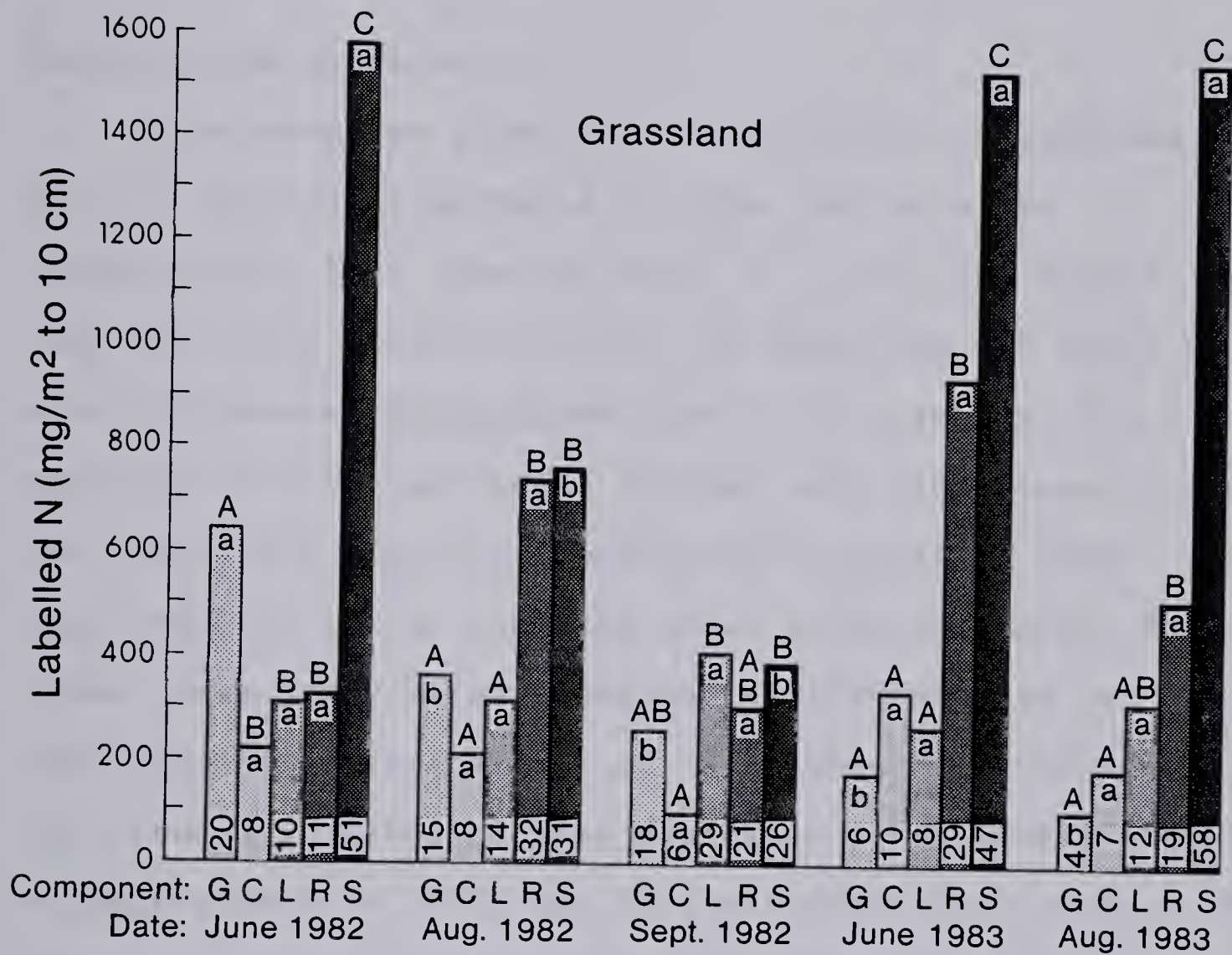


Fig. 11: Distribution of labelled N among shoots(G), crowns(C), litter(L), roots(R) and soil(S) at the native grassland. See Figure 8 for a complete description.

1982, and in 1983, was recovered in the soil. The proportion of labelled N in the root component varied from 11% to 32% and with a few exceptions, aboveground components contained a relatively small proportion of labelled N.

Between-site Differences

There were few significant differences in labelled shoot N between sites (Table 8). The 1980 site had significantly less labelled shoot N in June and August 1982 than the other study sites but, as described for total N, site differences disappeared later in the season. The quantity of N in the crowns did not vary significantly among the sites. The 1980 site consistently contained less labelled N in litter than the other reclaimed areas. The other three study sites contained similar amounts of labelled N in litter except in June 1982. Few consistent differences in labelled root N between sites were evident. At peak growth in 1982, the 1974 site had significantly more labelled N in its roots than any of the other sites.

The majority of labelled N in the system was found in the soil of the 1980 and grassland sites although this was not consistent in the latter site throughout the study. Roots were also a major sink for labelled N in the native grassland in August 1982 and June 1983. At the 1977 and 1974 sites labelled N tended to be distributed uniformly among plant and soil components in 1982 but by 1983, soil and root components contained over half the labelled N.

Table 8. Quantity of labelled nitrogen in components of the four study sites (mg/m² to 10 cm)

| DATE | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
|---------------|-------------------|--------------|--------------|---------------|
| SHOOTS | | | | |
| June 1982 | 178a ¹ | 698b | 472ab | 693b |
| Aug. | 190a | 635b | 513bc | 361ac |
| Sept. | 522a | 497a | 449a | 252a |
| June 1983 | 163a | 77a | 112a | 179a |
| Aug. | 145ab | 123ab | 181b | 108a |
| CROWNS | | | | |
| June 1982 | 166a | 299a | 395a | 218a |
| Aug. | 331a | 602a | 298a | 205a |
| Sept. | 295a | 272a | 141a | 101a |
| June 1983 | 290a | 209a | 403a | 330a |
| Aug. | 268a | 264a | 200a | 185a |
| LITTER | | | | |
| June 1982 | 7a | 225b | 327c | 316c |
| Aug. | 34a | 399b | 319b | 312b |
| Sept. | 10a | 237b | 184b | 405c |
| June 1983 | 136a | 435b | 455b | 264ab |
| Aug. | 130a | 559b | 514b | 316b |
| ROOTS | | | | |
| June 1982 | 152a | 278b | 188a | 329b |
| Aug. | 289a | 544ab | 1160c | 747b |
| Sept. | 422a | 313a | 258a | 305a |
| June 1983 | 276a | 722b | 608ab | 947b |
| Aug. | 188a | 425b | 525b | 515b |
| SOIL | | | | |
| June 1982 | 1240a | 248b | 265b | 1570c |
| Aug. | 838a | 356a | 514a | 766a |
| Sept. | 1260a | 452b | 382b | 388b |
| June 1983 | 798a | 447a | 773a | 1540b |
| Aug. | 1390a | 598b | 481b | 1570a |

1. Different letters indicate significant differences ($p \leq 0.05$) in component N between sites on any one date.

DISCUSSION

The small quantity of N in each of the plant components on the 1980 site relative to the other study sites, reflected its early stage of development. The quantities of N in each of the shoot, crown and root components on the 1974 site were similar to the amounts measured in each of those components on the 1977 site. This suggests that the amounts of N in living plant components on the reclaimed sites reached a stable maximum within 5 years of site establishment.

The amount of N measured in shoots on the grassland site were similar to those measured on the 1977 and 1974 sites, but this may have been due to an underestimate of grassland shoot N. Periodic measurement of standing crop yields an estimate of minimum shoot production because shoot growth and senescence occur continuously over the growing season (Risser *et al.* 1981). Although the underestimation was common to all study sites, the greater species diversity on the grassland resulted in a more significant underestimation than on the reclaimed sites. The large increases in quantities of litter N on the grassland observed in September 1982 and August 1983, therefore suggested that gross shoot production and shoot N were higher on this site than on the reclaimed areas despite similarities in standing crop.

Although the 1974 site is older than the 1977 site, the additional four years of litter production had resulted in a

relatively small increase in surface litter accumulation and indicated a near steady-state litter N component on the 1974 site. The litter component was therefore not a continuing sink for N, rather, through decomposition it returned N to the soil. Accumulation of N within reclaimed systems over the long term must therefore be in the soil rather than in plant materials. The native grassland was assumed to be at steady-state and therefore the amount of litter N, and the proportion of total plant N which this component represented, were stable, and were higher than in any of the reclaimed sites. The accumulation of N in litter of the reclaimed systems could be expected to continue until this steady-state condition was reached, without impeding the return of N from plant residues to the soil.

Large amounts of root N measured in the native grassland compared to the reclaimed sites suggested that root N increased with site age. Such a trend was not clear among the reclaimed sites. The quantities of N in the root components of the 1977 and 1974 sites were generally greater than in the 1980 site, but no increase in root N with site age could be distinguished between the older reclaimed areas because of high variability in root biomass within these sites. The 1974 site seemed to produce more roots during the peak period in August but differences at the other sampling dates were less dramatic.

Amounts of root N were two to three times higher in a subalpine grassland located about two km from the present

study sites, (Ziemkiewicz 1979), than those found on the native grassland in this study. Roots were sampled to a maximum depth of 24 cm compared to 10 cm in the present study, and this may account, in part, for the discrepancy between the two grasslands. It is apparent that a 10 cm sampling depth significantly underestimates root production in natural grasslands. It was, however an adequate depth for assessment of root growth in reclaimed sites. Roots were observed to be concentrated in the upper 10 cm of soil in the reclaimed sites and, although present below this depth, growth was sparse. If total root growth, rather than surface root growth, had been measured in the study sites, root biomass in the native grassland would have far exceeded that of the nine year old reclaimed site. It is possible that roots develop initially in the surface soil layer of the reclaimed sites, and penetrate lower horizons as site development proceeds (Fyles 1980).

The continuous increase observed in shoot N over the growing season in the 1980 site was not evident in any of the other sites and may be a result of species differences between the sites. The 1980 site was dominated by timothy, a mid to late-season grass; while the 1977 and 1974 sites had high proportions of orchardgrass and smooth brome, both early season grasses (Appendix II). The observations are consistent with continued timothy yield increase later into the growing season than would be expected with the other two species.

The decline in shoot N measured between August 1982 and August 1983 on the reclaimed sites was due to low precipitation early in the 1983 growing season retarding shoot production (Appendix I). Samples taken later in the 1983 season from these sites indicated that maximum standing crop was similar to that measured in August 1982.

The proportion of total plant N measured in each component varied substantially between years and during the growing season. The onset of flowering and peak standing crop of roots occurred in August 1982 on the three oldest sites. At this time, the majority of plant N was in the roots at all except the 1980 site. The crown component was the dominant sink for N on the 1980 site and was also important in the 1977 site. Seed production and peak standing crop of shoots occurred in mid-September on all sites in 1982. At this date, most plant N was in the roots of plants growing on the 1980 site, the roots and litter of the grassland and was fairly evenly distributed among all the plant components on the 1977 and 1974 sites. This demonstrated that significant changes in the distribution of N within the plant system occurred during the growing season.

The proportion of labelled N recovered in the system on the final sampling date, almost 15 months after application, indicated that added N losses were low in all sites. The fluctuations in % recovery of labelled N within the soil/plant system during the study were not easily

explained. A possible source of variability was some inconsistency in the depth to which samples were taken. Compacted areas and large rocks frequently made it impossible to obtain the desired 10 cm depths in all parts of the sample, potentially reducing the amount of labelled N recovered.

Low recovery of labelled N in plants growing on the 1980 site was a result of low plant yield, and consequently a low nutrient uptake capacity. The high total recovery of labelled N in this site compared to the low plant recovery, indicated that almost 50% of the labelled N recovered in the system remained in the soil throughout the study period. Losses of labelled N, however, were small and it is possible that compaction of the spoil material prevented rapid downward percolation of water and substantially reduced leaching losses.

The efficiency with which plants on the two older reclaimed sites and the grassland used the applied N reflected the large root biomass and high N requirements in these sites. The important residual effect of fertilizer was demonstrated in 1983, one year after initial application, when up to 56% of the labelled N was recovered in the plants.

A net increase or decrease in the amount of labelled N in any one component was not necessarily balanced by a measureable, equal and opposite change in another component. This is due, in part, to the dynamic nature of the N cycle

in which gains and losses of N occur simultaneously and only net changes are measured. It may also be a result of varying amounts of total fertilizer recovered in the system which masked changes occurring between dates.

Following application, the majority of the fertilizer taken up into the plant in all sites moved into the actively growing shoots and the crowns. The labelled N present in the litter in June can be attributed partly to fertilizer initially immobilized in the high C:N ratio surface plant material, and partly to adsorption (Clark *et al.* 1975). As the season progressed, root growth resulted in an increase in the amount of labelled N in the roots. Such a surge of labelled N uptake was not countered by a drop in labelled soil N except on the native grassland. The increase in labelled root N on the reclaimed sites coincided with an increase in total recovery, and it is possible that labelled N, which leached below the sampling zone in June, was taken up by the growing roots and therefore recovered in the August sample. This could explain the absence of an expected decline in labelled soil N and the overall increase in total % recovery.

By mid-September, the roots died back and presumably some of the labelled N previously contained in them entered the soil component. There was no measurable increase in labelled N in the soil at this time, possibly due to N released from the roots leaching beyond the sampling zone.

In the year following application, it was evident that the pulse of labelled N which had initially moved into the shoots had been transferred to the litter component. The root component continued to be a major sink for labelled N in the plant except on the 1980 site where the crown component dominated.

The pattern of change in labelled soil N at the grassland site over the study period was unique among the study sites. The drop in labelled N in the soil between June and August was attributed to increased plant uptake particularly into the root component, and of some leaching below the sampling zone. By June of the following growing season, the amount of labelled soil N increased dramatically indicating that much of the fertilizer which was previously in the plant had returned to the soil. Such rapid recycling of labelled N was not as evident in the reclaimed sites.

The apparently constant amount of labelled N in the crown component observed in this study was also reported in a shortgrass prairie by Clark (1977). The constancy may be due either to labelled N inputs equalling outputs or the capacity of the crowns to retain N but, because only net changes were measured, these mechanisms could not be distinguished.

B. TURNOVER AND FLOWS OF LITTER NITROGEN

It was suggested in an earlier section that the litter component of the older reclaimed sites was approaching a steady state, and it was evident that decomposition and flow of N through litter were occurring. The rate of litter N turnover, and the amount of N transferred from the litter component to the soil or lost from the system, was compared between sites.

Flow of N from surface litter between two successive sampling dates was estimated as follows: A decline in labelled shoot N ($\Delta\text{SHOOTNL}$) between sampling dates was assumed to be a consequence of shoot material dying and therefore represented an input of labelled N to the litter component. It was also assumed that the ratio of labelled N to total N (NL:NT) in material added to the litter, was equal to the NL:NT ratio of the shoots, so that additions of total N ($\Delta\text{SHOOTNT}$), could be calculated from the measured additions of labelled N. Inputs of shoot N to the litter component, and net changes in litter N between two sampling dates were made as follows:

$$(\Delta\text{SHOOTNL})/(\text{NL:NT of shoots})=\Delta\text{SHOOTNT}$$

$$(\Delta\text{SHOOTNL}+\text{LITTERNL}_{t_1})-(\text{LITTERNL}_{t_2})=\text{LITTERNL}_{t_1 \text{ to } t_2}$$

$$(\Delta\text{SHOOTNT}+\text{LITTERNT}_{t_1})-(\text{LITTERNT}_{t_2})=\text{LITTERNT}_{t_1 \text{ to } t_2}$$

At the start of the winter period (September 1982), all shoot N minus that retranslocated, entered the litter component. In this study, samples were not collected sufficiently late into the fall to assess the proportion of N retranslocated to roots and crowns before senescence occurred. However, N concentration in shoot samples collected from the reclaimed areas in the fall of 1979, declined 31% between mid-September and mid-October. This estimate of translocation was similar to the average calculated by Clark (1977) and for the purposes of this study, it was assumed that 30% of the N in the shoots was translocated back into the plant's storage organs before shoot material entered the litter component.

Flows of labelled or total litter N which occurred during each of the four sampling intervals are reported for each plot in Appendix VII. The sum of these flows, or total litter N flow for the 15 month study period, was converted to an annual basis after multiplying each flow by 12/15 (Table 9).

The turnover of labelled and unlabelled N was calculated by dividing total flows by the average quantity of labelled or total litter N measured on the sites. Turnover rates were calculated for each replicate and mean rates for the sites were reported on an annual basis as described for total flows (Table 9). Turnover calculations required that the labelled and total N in the litter be at steady state. Although this held for the duration of the

Table 9. Turnover and release of litter nitrogen on four subalpine sites at Westar Mining Ltd.

| <u>SITE</u> | <u>ANNUAL N FLOW</u> | | <u>AVERAGE AMOUNT OF LITTER N</u> | | <u>TURNOVER RATE</u> | |
|---------------|-----------------------------------|-----------|---------------------------------------|-----------|--------------------------|-----------|
| | <u>NL</u> (mg/m ²) | <u>NT</u> | <u>NL</u> (mg/m ²) | <u>NT</u> | <u>NL</u> (1/y) | <u>NT</u> |
| 1980 | 274a ¹ | 478a | 133a ² | 422a | 2.12a | 1.11a |
| 1977 | 628b | 1990b | 371b | 2360b | 1.70ab | 0.86a |
| 1974 | 426ab | 3760b | 379b | 3840c | 1.14b | 0.97a |
| GRASS LAND | 612b | 7340c | 322b | 6880d | 1.88a | 1.04a |

1. Different letters indicates significant differences (p≤0.05) between sites.

2. Only 1983 data were used to calculate average quantity of N in litter of the 1980 site. In all other sites 1982 and 1983 data were averaged.

study on the three oldest study sites, it was evident that it was not true on the 1980 site. Total and labelled litter N tripled during the study period on this site, (as reported in Figures 3 and 7), and therefore an average of the 1983 litter N values was used in the turnover rate calculation. It was recognized that such a procedure underestimated turnover rate of litter N on this site.

RESULTS

The release of total N from the litter component during the study period was highest on the native grassland and lowest on the 1980 site (Table 9). There was no significant difference between the litter N released on the 1977 and 1974 sites. No significant difference in flow of labelled N was measured between the three oldest sites and values for the 1980 site were significantly lower than on the 1977 and grassland sites but not the 1974 site.

On the 1980, 1977 and native grassland sites, the turnover rate of labelled N was significantly higher than the respective rates of total N turnover (Table 9). No such difference between the two rates was observed on the 1974 site. Between sites, there were no significant differences in total N turnover rates but labelled N recycled more slowly on the 1974 site than on the 1980 or grassland sites.

DISCUSSION

Substantial amounts of N were released by the litter component of the three oldest sites during the 15 month study period. The amount of N which flowed through the litter on the 1977 and 1974 sites averaged 25 and 47 kg/ha of N, respectively. The 1977 value was within the range of litter N flows reported for the Cottonwood mixed-grass prairie and the 1974 value was near the maximum reported for the Osage tallgrass prairie (Bokhari and Singh 1975). On the native grassland, the flow of N from the litter averaged 92 kg/ha and far exceeded the flows measured in prairie systems. Such results are due to the large amount of N in the litter at this site.

Considerable release of N occurred during the winter months. On average, 20 to 30% of the total litter N released in the 1974 and 1977 sites, and 74% of that released in the native grassland, occurred between mid-September 1982 and early June 1983. Values from the native grassland were comparable to the over-winter decrease in litter biomass reported by Knight and Kyte(1975) and Bleak(1970) in subalpine grasslands. The difference between the reclaimed and native sites in the size of N flows from the litter in winter may be due in part, to the early disappearance of snow from the native, southwest-facing grassland and the occurrence of rapid litter decomposition in the weeks before the June sample was collected. The snow had only recently melted from the reclaimed sites by June 1 so that a similar

period of warm, moist conditions had not occurred.

Turnover rates of litter N were rapid on the reclaimed and native high elevation grassland systems. The high turnover rates of labelled N relative to total N on three out of four sites demonstrated that N in recently added plant material recycled more quickly than more stabilized and humified residues. Rapid decomposition of fresh plant litter, with conversion of a portion to more resistant forms, is well-documented in the literature (for example: Hunt 1977, McGill *et al.* 1981, Weider *et al.* 1983). Clark (1977) reported that the turnover rate of labelled litter N on a shortgrass prairie was between 0.50 and 0.70 1/y. His estimate is less than half that measured on the reclaimed and native grassland sites in this study.

Rapid turnover of litter N on the study sites not only indicates the dynamic nature of surface litter but also shows that N recycles more quickly through litter on these reclaimed systems than on self-sustaining prairie grasslands. Rapid recycling of litter on high elevation grasslands may be due, in part, to moist, favourable conditions for decomposition which occur in winter under snow.

C. DYNAMICS OF COARSE AND FINE ROOT NITROGEN

Coarse and fine roots were separated in this study in an attempt to distinguish two physically distinct root components which might cycle N at different rates. The

amount and rate of N recycled by each of these components was compared between sites to assess possible changes in the contribution of coarse and fine roots to N cycling as site age increased.

DIFFERENCES BETWEEN COARSE AND FINE ROOTS

RESULTS

Nitrogen concentration was higher in fine root fragments than in coarse root fragments at most sampling dates but not all the differences were statistically significant (Table 10).

On an area basis, coarse root total N was higher than fine root total N only in the 1980 site (Table 11). These results reflected the consistently higher coarse root biomass relative to fine root biomass on this site. On the native grassland, in 1983 only, total N in the fine roots was significantly higher than that in the coarse roots. With the exception of June 1982, coarse and fine root fragments contained similar amounts of N at the 1977 and 1974 sites.

Labelled N content followed the same pattern as total N content in all sites (Table 12). Labelled N in coarse root fragments on the native grassland was significantly higher than that of fine roots in June and August 1982, and was higher, although not statistically different, than fine root labelled N in September. In the year following, the distribution changed and fine root fragments contained

TABLE 10. NITROGEN CONCENTRATION(%) IN COARSE AND FINE ROOTS AT FOUR SUBALPINE SITES AT WESTAR MINING LTD.

| DATE | 1980 SITE | | 1977 SITE | | 1974 SITE | | GRASSLAND | |
|-----------|--------------------|-------|-----------|-------|-----------|-------|-----------|-------|
| | COARSE | FINE | COARSE | FINE | COARSE | FINE | COARSE | FINE |
| June 1982 | 0.67a ¹ | 0.66a | 0.63a | 0.72b | 0.84a | 0.83a | 1.20a | 1.51b |
| Aug | 0.58a | 0.74a | 0.57a | 0.59a | 0.72a | 0.69a | 1.22a | 1.31a |
| Sept. | 0.52a | 0.61a | 0.43a | 0.50a | 0.54a | 0.69b | 1.15a | 1.50b |
| June 1983 | 0.52a | 0.72b | 0.59a | 0.76b | 0.60a | 0.76a | 1.07a | 1.36b |
| Aug. | 0.55a | 0.70b | 0.52a | 0.68b | 0.57a | 0.75b | 0.86a | 1.29b |

1. Different letters indicate significant differences ($p \leq 0.05$) between coarse and fine roots at any site on any one sampling date.

TABLE 11. QUANTITY OF NITROGEN IN COARSE AND FINE ROOTS ON THE FOUR STUDY SITES (mg/m² to 10 cm).

| DATE | 1980 SITE | | 1977 SITE | | 1974 SITE | | GRASSLAND | |
|-----------|-------------------|------|-----------|-------|-----------|-------|-----------|-------|
| | COARSE | FINE | COARSE | FINE | COARSE | FINE | COARSE | FINE |
| June 1982 | 639a ¹ | 98b | 3080a | 2220a | 2750a | 1020b | 4100a | 3000a |
| Aug. | 1060a | 172b | 2950a | 3550a | 5150a | 4370a | 7230a | 5040a |
| Sept. | 1390a | 275b | 1070a | 1650a | 2400a | 2180a | 4430a | 3840a |
| June 1983 | 622a | 270b | 2570a | 3320a | 2620a | 3530a | 2960a | 7780b |
| Aug. | 663a | 90b | 2270a | 2340a | 2340a | 2340a | 3220a | 8080b |

TABLE 12. QUANTITY OF LABELLED NITROGEN IN COARSE AND FINE ROOTS ON THE FOUR STUDY SITES (mg/m² to 10 cm)

| DATE | 1980 SITE | | 1977 SITE | | 1974 SITE | | GRASSLAND | |
|-----------|-------------------|------|-----------|------|-----------|------|-----------|------|
| | COARSE | FINE | COARSE | FINE | COARSE | FINE | COARSE | FINE |
| June 1982 | 130a ¹ | 21b | 159a | 119a | 151a | 36b | 239a | 90b |
| Aug. | 249a | 40b | 258a | 287a | 609a | 555a | 462a | 285b |
| Sept. | 346a | 77b | 138a | 175a | 154a | 104a | 188a | 116a |
| June 1983 | 195a | 81b | 364a | 358a | 295a | 314a | 312a | 635b |
| Aug. | 167a | 21b | 182a | 243a | 333a | 192a | 167a | 348b |

1. Different letters indicate significant differences ($p \leq 0.05$) between coarse and fine roots at any one site and on any one sampling date.

significantly more labelled N than coarse roots fragments. Similar changes were evident on the 1974 site but they were not statistically significant.

The proportion of total N (NT) in the roots which was derived from labelled N (NL), the NL:NT ratio (Table 13). No significant differences between the NL:NT ratios of coarse and fine root fragments were measured in any sites. The NL:NT ratios of roots at the 1980 site were significantly higher than at any of the other study sites. No statistically significant differences were measured between the other three sites in the NL:NT ratios of coarse and fine root fragments, although a decrease in NL:NT ratio of fine root fragments occurred as site age increased.

DISCUSSION

Although coarse and fine roots were separated on the basis of physical, rather than functional criteria, higher N concentrations in fine roots relative to coarse roots may be a result of functional differences between the two root components. Two origins of such differences can be proposed. First, fine root fragments may contain a higher proportion of actively growing tissue than coarse roots which would result in a high average N concentration. Coarse roots would therefore be expected to have a higher proportion of structural material and therefore a lower concentration of N. Alternatively, the fine root component may contain a larger proportion of dead, decaying material than the coarse

TABLE 13. PROPORTION OF TOTAL NITROGEN DERIVED FROM LABELLED NITROGEN IN COARSE AND FINE ROOTS(NL:NT RATIO)

| DATE | 1980 SITE | | 1977 SITE | | 1974 SITE | | GRASSLAND | |
|-------------|-------------------|--------|-----------|--------|-----------|--------|-----------|--------|
| | COARSE | FINE | COARSE | FINE | COARSE | FINE | COARSE | FINE |
| June 1982 | 0.20 ¹ | 0.21 | 0.05 | 0.05 | 0.05 | 0.04 | 0.06 | 0.03 |
| Aug. | 0.24 | 0.23 | 0.09 | 0.08 | 0.12 | 0.13 | 0.06 | 0.06 |
| Sept. | 0.25 | 0.28 | 0.13 | 0.11 | 0.06 | 0.05 | 0.04 | 0.03 |
| June 1983 | 0.32 | 0.30 | 0.14 | 0.11 | 0.11 | 0.09 | 0.11 | 0.08 |
| Aug. | 0.25 | 0.23 | 0.08 | 0.10 | 0.14 | 0.08 | 0.05 | 0.04 |
| Average | | | | | | | | |
| NL:NT ratio | 0.25ax | 0.25ax | 0.10ay | 0.08ay | 0.10ay | 0.08ay | 0.06ay | 0.05ay |

1. Different letters (a,b) indicate significant differences ($p \leq 0.05$) between coarse and fine roots at any one site and on any one sampling date. Different letters (x,y) indicate significant difference ($p \leq 0.05$) between sites in any component.

root component. This material would have a relatively high N concentration due to microbial immobilization of N and respiratory loss of C, producing a high N concentration in the fine roots.

Decreasing NL:NT ratios of coarse and fine root fragments with site age, and higher ratios in coarse root relative to fine root fragments in the older sites, support the latter explanation. During early site development, coarse and fine root fragments are of recent origin and therefore have similar NL:NT ratios. Over time, coarse root fragments die and are comminuted, yielding fine root fragments. Immobilization of N by organisms in fine root fragments enriches them in total N and, as decomposition proceeds, humified and resistant components develop. Consequently, as site age increases, dilution of labelled N by N contained in resistant material is greater in fine root fragments than in coarse root fragments. A greater decrease in NL:NT ratio should therefore occur in the fine root than in coarse root fragments as site age increases. The data in Table 13 are consistent with this hypothesis. Accumulation of total and labelled N in fine root fragments of the native grassland and 1974 sites during the study also support this hypothesis (Tables 11 and 12).

ROOT NITROGEN RELEASE AND TURNOVER

The substantial decline in coarse and fine root biomass which occurred between August and September 1982 on the

1977, 1974 and grassland sites, was attributed to annual, late season senescence. The amount of N released from the roots during this dieback provided an estimation of the amount of root N recycled annually. Because no decline in root biomass was measured on the 1980 site, no estimation of root turnover could be made.

The quantity of total N (unlabelled N+labelled N), released from the coarse and fine roots was equal to the difference between the amount of total N present in each component in August 1982 and the amount present in September 1982. The flow of labelled N was calculated in a similar manner. The turnover of total and labelled N in the coarse and fine roots was calculated by dividing the quantity of total or labelled N released from each component during the August to September period, by the amounts present in August. Turnover rates were calculated for each replicate and averaged to obtain a mean rate for each study site.

RESULTS

Because mid-September to late October losses of root N were not measured, and the effects of fertilization on root dynamics and annual variation were unknown in this study, the calculated values are only a first approximation of annual root N flow at high elevation native and reclaimed grasslands.

There was no significant difference between the coarse and fine root fragments, in the amount of labelled or total

N released in any of the study sites (Table 14). No significant differences in the total amount of N released by the roots were observed between sites. Roots in the 1974 site released more labelled N than in the 1977 and grassland sites but this difference was significant only in fine root fragments.

Turnover rates of total and labelled N did not differ between the two root components (Table 14). The only between-site difference in the rate of labelled or total N turnover occurred between the 1974 and 1977 sites with fine root fragments at the 1974 site recycling labelled N faster than at the 1977 site.

DISCUSSION

The large amount of N lost from the coarse and fine roots between August and September 1982, demonstrated the importance of the root component to N cycling. Total N released from both root components was 38, 51 and 49 kg/ha in the 1977, 1974 and grassland sites, respectively. Root N released in the 1977 site was similar in magnitude to that reported for the Osage tallgrass prairie (Table 4), (Bokhari and Singh 1975, Woodmansee *et al.* 1981), and for a mesic prairie in central Missouri (Dahlman and Kucera 1965). Values for root N release in the 1974 and grassland sites exceeded all values reported in the literature possibly because few of these data were derived using ^{15}N and other methods may not have been as sensitive in measuring root

TABLE 14. TURNOVER AND RELEASE OF COARSE AND FINE ROOT NITROGEN

| SITE | NITROGEN RELEASED BETWEEN AUGUST AND SEPTEMBER 1982 | | | | RATE OF ROOT NITROGEN TURNOVER | | | |
|-----------|--|------|---------|-------|--------------------------------|--------|---------|-------|
| | LABELLED N | | TOTAL N | | LABELLED N | | TOTAL N | |
| | COARSE | FINE | COARSE | FINE | COARSE | FINE | COARSE | FINE |
| | ----- (mg/m ² to 10 cm) ----- | | | | ----- (1/y) ----- | | | |
| 1977 | 120a | 113a | 1873a | 1906a | 0.44a | 0.42a | 0.64a | 0.52a |
| 1974 | 437a | 451b | 2751a | 2372a | 0.49a | 0.82b | 0.49a | 0.53a |
| Grassland | 292a | 169a | 3173a | 1739a | 0.53a | 0.55ab | 0.56a | 0.32a |

1. Different letters indicate significant differences ($p \leq 0.05$) in component N between sites.

turnover.

The average turnover rate of root N, measured in all the sites was $0.51 \pm 0.30 \text{ y}^{-1}$. Within each site, however, root turnover rates changed dramatically over small distances, demonstrating the tremendous variability in rate of below-ground processes in the systems studied. A comparable range (0.22 to 0.74 y^{-1}) of turnover rates was reported by Sims and Singh (1978) for the shortgrass prairie. Other root turnover rates reported in the literature for arctic, tallgrass prairie and shortgrass prairie systems (Singh and Gupta 1977, Clark 1977), were generally lower than values measured here.

D. PROPORTION OF PLANT NITROGEN DERIVED FROM LABELLED NITROGEN

Of the total N (NT) in the living plant (shoots+crowns+roots) or in its parts, the proportion derived from labelled N (NL), referred to as NL:NT ratio, reflected plant growth responses to, or dependence on fertilizer inputs.

The significance of the NL:NT ratio of each plant component is a function of the component's longevity. Because the shoot component does not maintain a pool of stored N over winter, the NL:NT ratio of shoot N reflected the NL:NT ratio of N taken up from the soil and that retranslocated from storage in roots and crowns. Shoot N from the latter source was not distinguished in this study

but it was assumed to be a uniform proportion of total shoot N in all sites. In relatively long-lived plant components, such as the crowns and roots, the NL:NT ratio is a function of the amount and turnover rate of unlabelled N stored in these components prior to labelled fertilizer application.

RESULTS

Total Plant

The proportion of N in the total plant which was derived from labelled N did not change significantly during the study. For this reason, an average value for each site was calculated for the entire study period (Figure 12). The NL:NT ratio in the plants of the 1980 site was significantly higher than in the other three sites. No significant difference was measured between the 1977 and 1974 sites, and the native grassland had a significantly lower NL:NT ratio than any of the other study sites.

Shoots

The NL:NT ratio of shoot N on the 1980 site did not change throughout the 1982 and 1983 growing seasons (Table 15). On the 1977 site the ratio did not change during 1982 but decreased significantly in 1983. Ratios declined significantly between June and September 1982 on the 1974 and grassland sites, and decreased further by August 1983. In June and August 1982, there was no statistically significant difference in the NL:NT ratio of the shoots between the three reclaimed sites. For the remainder of the study period, ratios for the 1980 site were significantly higher than at the other study sites. There was no significant difference in shoot NL:NT ratio between the 1977 and 1974 sites after June 1982. The grassland shoots had the smallest NL:NT ratios at all sampling dates but these

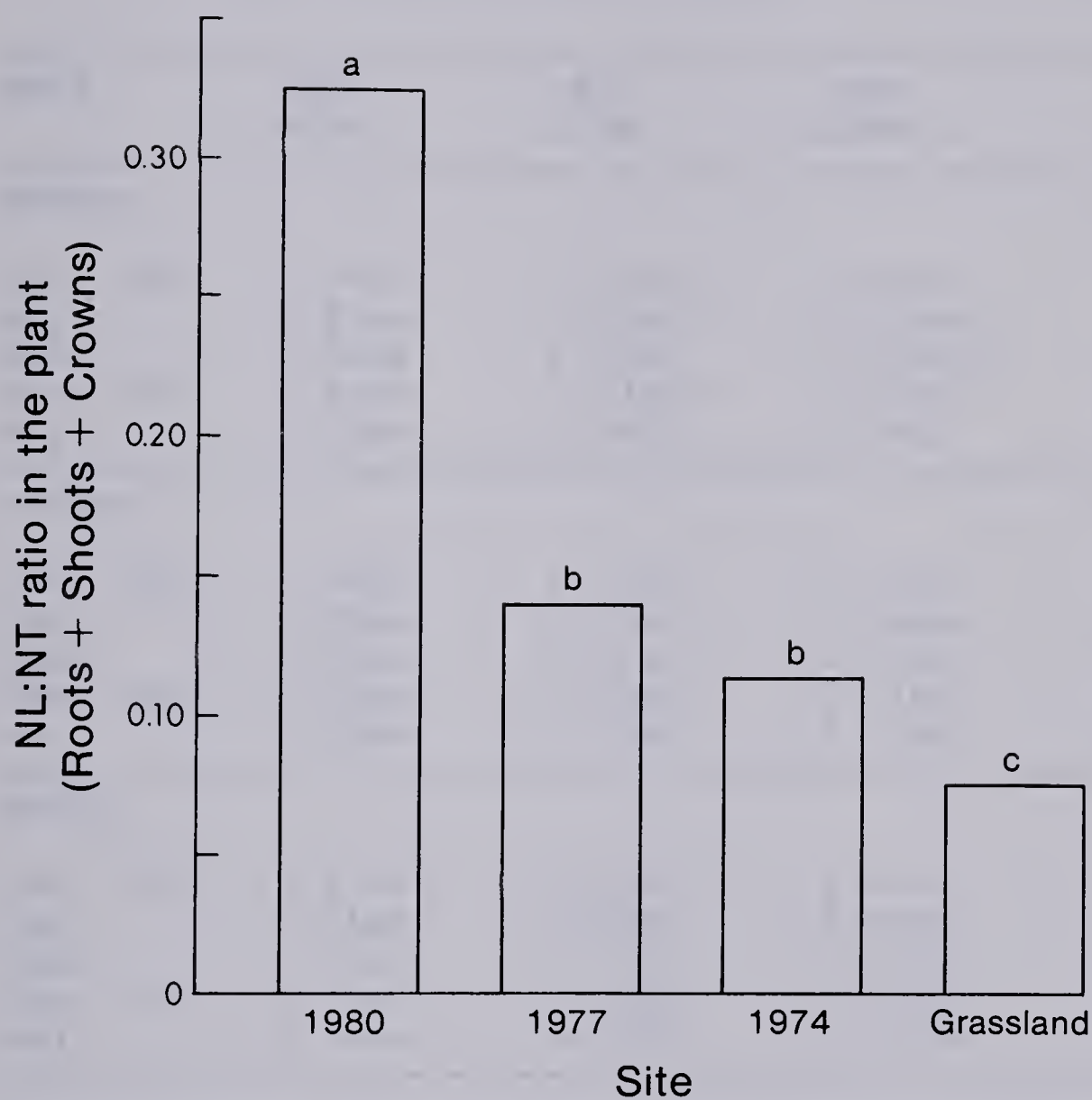


Fig. 12: The NL:NT ratio of living plants (shoots + crowns + roots) at the four study sites. Different letters indicate significant differences ($p \leq 0.05$) between sites.

Table 15. Proportion of total nitrogen derived from labelled nitrogen in plants(NL:NT ratio) at the four study sites.

| DATE | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
|---------------|---------------------|--------------|--------------|---------------|
| SHOOTS | | | | |
| June 1982 | 0.44ax ¹ | 0.35axy | 0.42ax | 0.22ay |
| Aug. | 0.47ax | 0.37axy | 0.32abxy | 0.21ay |
| Sept. | 0.56ax | 0.33ay | 0.24bcyz | 0.14bz |
| June 1983 | 0.48ax | 0.27aby | 0.26bcy | 0.11bz |
| Aug. | 0.42ax | 0.20by | 0.19cy | 0.05cz |
| CROWNS | | | | |
| June 1982 | 0.28ax | 0.11ay | 0.10ay | 0.07ay |
| Aug. | 0.28ax | 0.12ay | 0.14ayb | 0.08ay |
| Sept. | 0.39ax | 0.18ay | 0.07ay | 0.06ay |
| June 1983 | 0.35ax | 0.15ay | 0.11ay | 0.10ay |
| Aug. | 0.34ax | 0.11ay | 0.13ay | 0.08ay |
| ROOTS | | | | |
| June 1982 | 0.21ax | 0.05ay | 0.05ay | 0.05ay |
| Aug. | 0.24ax | 0.08ay | 0.12by | 0.06ay |
| Sept. | 0.25ax | 0.12ay | 0.06ay | 0.04az |
| June 1983 | 0.31ax | 0.12ay | 0.10aby | 0.09by |
| Aug. | 0.25ax | 0.08ay | 0.11abz | 0.05ay |

1. Different letters (a,b,c) indicate significant differences ($p \leq 0.05$) between dates at any one site and in any one component. Different letters (x,y,z) indicate significant differences ($p \leq 0.05$) between sites on any one date.

differences were statistically significant only in 1983.

Crowns

No significant seasonal changes occurred in the NL:NT ratio of crowns throughout the study in any of the sites (Table 15). Crowns in the 1980 site consistently had the highest NL:NT ratios of all the study sites, but no significant differences between the other sites were measured.

Roots

Roots on the 1980 and 1977 sites showed no change in NL:NT during the study (Table 15). The NL:NT ratio peaked in August 1982 and in 1983 on the 1974 site. A peak occurred in June 1983 on the native grassland, but ratios declined in August. Between-site differences followed a pattern similar to that described for the crowns.

A comparison of the average NL:NT ratios measured in each of shoot, crown and root component at the 1974 site is described in Figure 13. The plant components of the other sites showed a similar pattern of NL:NT ratios with the highest ratios measured in shoots and lowest ratios in roots.

DISCUSSION

A substantial decline in the NL:NT ratio of the living plant was evident as site age increased. The high ratio measured in the 1980 site demonstrated that during the first

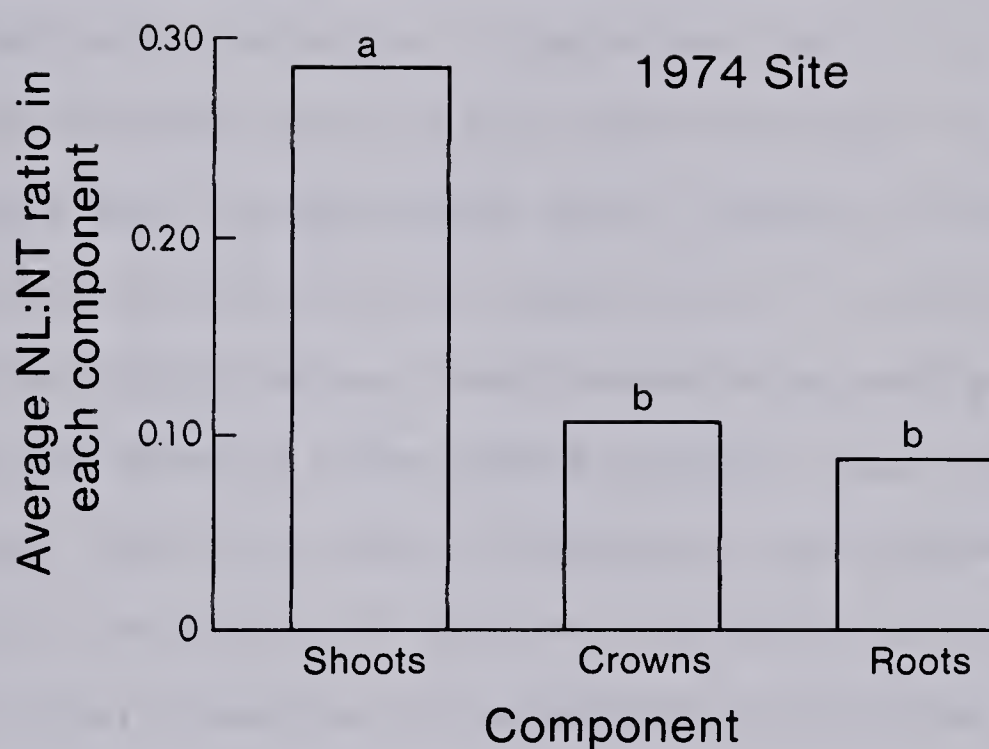


Fig. 13: The average NL:NT ratios of shoots, crowns, and roots at the 1974 site measured during the study. Different letters indicate significant differences ($p \leq 0.05$) between ratios.

two years after site establishment, plant productivity was dependent on annual inputs of fertilizer N. The high NL:NT ratio in plants growing on the young reclaimed site in 1983, reflected the importance of residual fertilizer N to plant growth in the year following application. In 1982 therefore, most of the N in the plants which did not come from uptake of the recently applied labelled fertilizer was probably derived from residual 1981 fertilizer.

The large decrease in the NL:NT ratio of plants which occurred between the two year and five year old sites, indicated that in the older site, organic N accumulated in the system during the five years prior to labelled fertilizer application, was mineralizing and providing unlabelled mineral N for plant uptake. Plant growth on the five year old site was considerably less dependent on annual inputs of fertilizer N than on the 1980 site. After five years, mineralization of unlabelled N continued to increase, and fertilizer dependence continued to decrease as site age increased, but these changes were less dramatic than during early stages of site development.

These results suggested that a supply of readily mineralizable N built up rapidly in the soil during the five years following site establishment, and increased slowly after this time. The longevity or stability of this active N fraction in reclaimed sites is a major control on the ability of each system to continue recycling N once fertilization is terminated. Data from the nine year old

site suggest that the active pool of N is maintained and mineralizes sufficient N to support plant growth without fertilizer inputs. This site was fertilized only one year longer than the five year old site, yet it produced plants with a similar NL:NT ratio to those from the five year site. It is therefore evident that N cycling processes have sustained a pool of readily mineralizable N of equal size to that which was present before management practices were terminated.

The very high NL:NT ratios in shoots compared to other plant components, demonstrated that shoots were the major sink for fertilizer N at least in the first growing season following application. The decline in shoot NL:NT ratios during 1982 on the 1974 and grassland sites indicated that the NL:NT ratio of N assimilated by shoots, had decreased over this period. This would occur as N mineralized from stabilized, unlabelled soil organic N increasingly diluted the labelled N mineralized from active organic N. Absence of a similar decline in shoot NL:NT ratio in the 1980 site, reflected the large amount of labelled soil N relative to unlabelled N available for plant uptake in this site throughout the 1982 and 1983 seasons. The small decline in the NL:NT ratio of shoots on the 1977 site, suggested that this site represented a transition between the young and old reclaimed sites, where mineralization of unlabelled N from a stable organic pool was sufficient to reduce the NL:NT ratio of the soil mineral N only slightly. These data indicate

that the development of a stabilized pool of organic N in the reclaimed sites lags behind that of the active pool.

The lower NL:NT ratio measured in the crown component relative to the shoot component was a function of the crown being a long-lived storage organ with a relatively slow rate of turnover. A small NL:NT ratio measured in the crowns of the three oldest sites reflected the dilution of the recently assimilated labelled N by unlabelled N stored prior to labelled fertilizer application. It was apparent that labelled N uptake was small relative to the amount of crown N in these sites. A high NL:NT ratio was measured in the crowns of the 1980 site and indicated that the crown component took up a larger amount of labelled N, relative to unlabelled N, than the older sites. The uptake of labelled N into the crowns supported the growth of new crown tissue, suggesting that the crown component on the 1980 site was increasing in size and had not yet reached steady state.

The NL:NT ratio was also high in the roots of the 1980 site and relatively low in the older sites for the same reason described for the crown component. The high NL:NT ratios of shoots relative to NL:NT ratios of other parts of the plant, and the significant decline in NL:NT ratio measured only in the shoots during the study period, indicated that N cycled more quickly through shoots than through crowns or roots. The relatively slow turnover of N in roots at the three oldest sites was reported previously, and is consistent with the hypothesis that roots in these

sites contain recalcitrant material. Similar NL:NT ratios measured in crowns and roots in the three oldest sites suggest that turnover rates are similar, and that crowns also contain material resistant to decomposition.

REDISTRIBUTION OF LABELLED NITROGEN AMONG THE PLANT COMPONENTS

The addition of labelled fertilizer to the reclaimed and natural grassland systems initially resulted in concentration of labelled N in growing tissues. Redistribution of labelled N among plant and soil components would, in the long term, result in a uniform NL:NT ratio throughout the system. A uniform distribution would be attained more rapidly in plant components than in the system as a whole, because the turnover rate of total N in soil is very slow. For this reason, and because the mine spoil contains N that may not participate in normal N cycling, redistribution of labelled N is meaningfully discussed only for the plant components.

Labelled N redistribution was described by calculating the variability obtained when averaging the NL:NT ratios of plant components of each study site, and at each sampling date. Wide variation in NL:NT ratios between sites resulted in a positive correlation between standard deviation and NL:NT ratio, necessitating the selection of a parameter which would eliminate the effect of ratio size. Use of the coefficient of variation as an index of variability removed

this effect.

At the first sampling date, the lowest variability was measured in the youngest reclaimed site (Table 16). This reflected the presence of growing tissue, actively assimilating labelled N, in each of the plant components. Higher variability, or less uniform distribution of labelled N was measured in the older sites due to low NL:NT ratios in those components composed largely of storage or senescent tissues, the crowns, roots and litter components, and the high NL:NT ratio in the actively growing shoots.

The decrease in variability measured on all sites during the 15 month study, indicated the redistribution of labelled N among plant components in response to nitrogen cycling processes.

E. SOIL NITROGEN MINERALIZATION

Soil organic matter can be described as two interacting fractions: a small, active and readily mineralizable fraction consisting of microorganisms and recent organic residues; and a larger, recalcitrant fraction (Hauck and Bremner 1976). Nitrogen is mineralized from both these fractions although the recalcitrant fraction mineralizes N more slowly and, over the short term, may make a smaller contribution to the net amount of N mineralized (Jansson 1975).

The amount of soil N mineralized during a 23 week laboratory incubation was compared between sites to assess

Table 16. Redistribution of labelled nitrogen among the plant components

| DATE | COEFFICIENT OF VARIATION | | | |
|-----------|--------------------------|--------------|--------------|---------------|
| | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
| June 1982 | 0.51 | 0.93 | 1.16 | 0.78 |
| Aug. | 0.35 | 0.73 | 0.60 | 0.60 |
| Sept. | 0.43 | 0.50 | 0.73 | 0.62 |
| June 1983 | 0.36 | 0.34 | 0.45 | 0.12 |
| Aug. | 0.23 | 0.46 | 0.26 | 0.29 |

changes in the capacity of the soils for net mineralization as site age increased. The proportion of mineralized N which was derived from labelled N was used to estimate the size of the active soil organic fraction in the study sites.

RESULTS

A curvilinear relationship was observed between the cumulative net amount of N mineralized and time of incubation (Figure 14). A negligible net amount of N was mineralized by soil from the 1977 site during the first 18 weeks of incubation and a small increase occurred during the final 5 weeks. Most of the net N mineralization (70 and 90% respectively) occurred in the first 8 weeks of incubation on the 1974 and 1980 sites. The cumulative net amount of N mineralized in the grassland soil increased throughout the incubation and far exceeded net mineralization in the reclaimed sites.

The proportion of N mineralized at each date which was labelled (NL:NT ratio), was highest in soil from the 1980 site and lowest in the native grassland soil (Figure 14). There was no statistically significant change throughout the incubation in the NL:NT ratio of N mineralized by soil from the 1974 site nor by the 1980 soil after week two. The NL:NT ratio of N mineralized by the native grassland soil declined continuously throughout the incubation.

The quantity of N in the active fraction was estimated using the relationship:

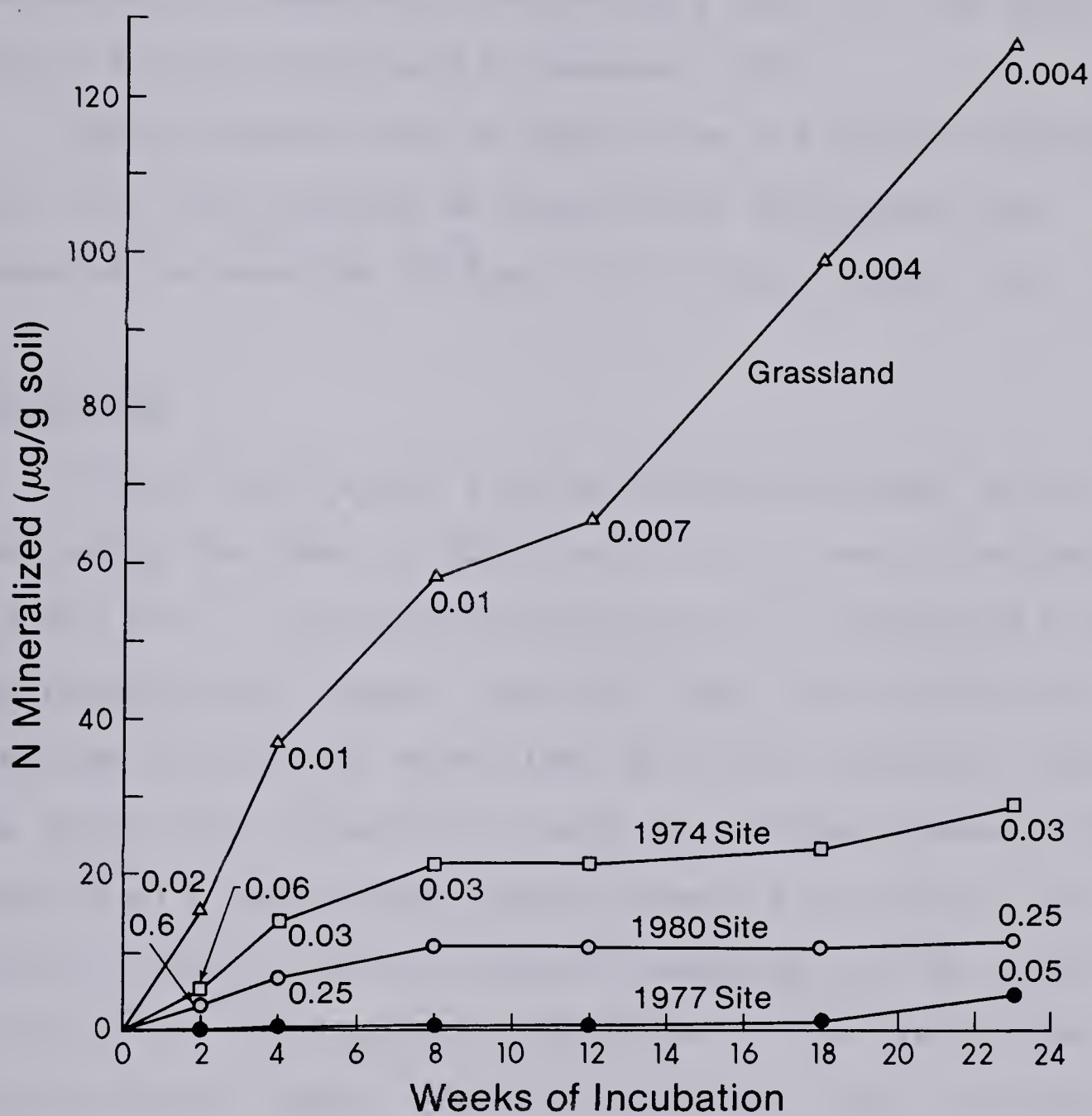


Fig. 14: Cumulative net amount of N mineralized during a 23 week incubation of soil from the four study sites. Numbers associated with points on the curves are NL:NT ratios of N mineralized at that date.

$$A=B(1-y)/y$$

where A is the amount of active organic N in the soil, B is the amount of labelled N in the soil and y is the NL:NT ratio of the mineralized N (Jansson 1975).

The estimated size of the active N fraction increased with site age although no significant difference was measured between the 1977 and 1974 sites (Figure 15).

DISCUSSION

Calculation of the y value differed between sites. The continuing decrease in NL:NT ratio with time of incubation was due to: i) increased contribution of unlabelled N from the recalcitrant organic fraction; and ii) transfer of labelled N into more stabilized fractions (Jansson 1975). The NL:NT ratio measured in week two of the incubation was considered to approximate most closely the ratio of the active N fraction at the time of sampling. It is likely that dilution and stabilization continued in the field after collecting the sample for the incubation study, and the A value in all sites would be expected to increase slowly over time.

No significant change in NL:NT ratio occurred in the soil from the 1974 site during the incubation and the y value was equal to the average of NL:NT ratios measured at all dates. In N mineralized from soil of the 1980 sites, the NL:NT ratio dropped after week two and stabilized for the duration of the incubation. The highly labelled N

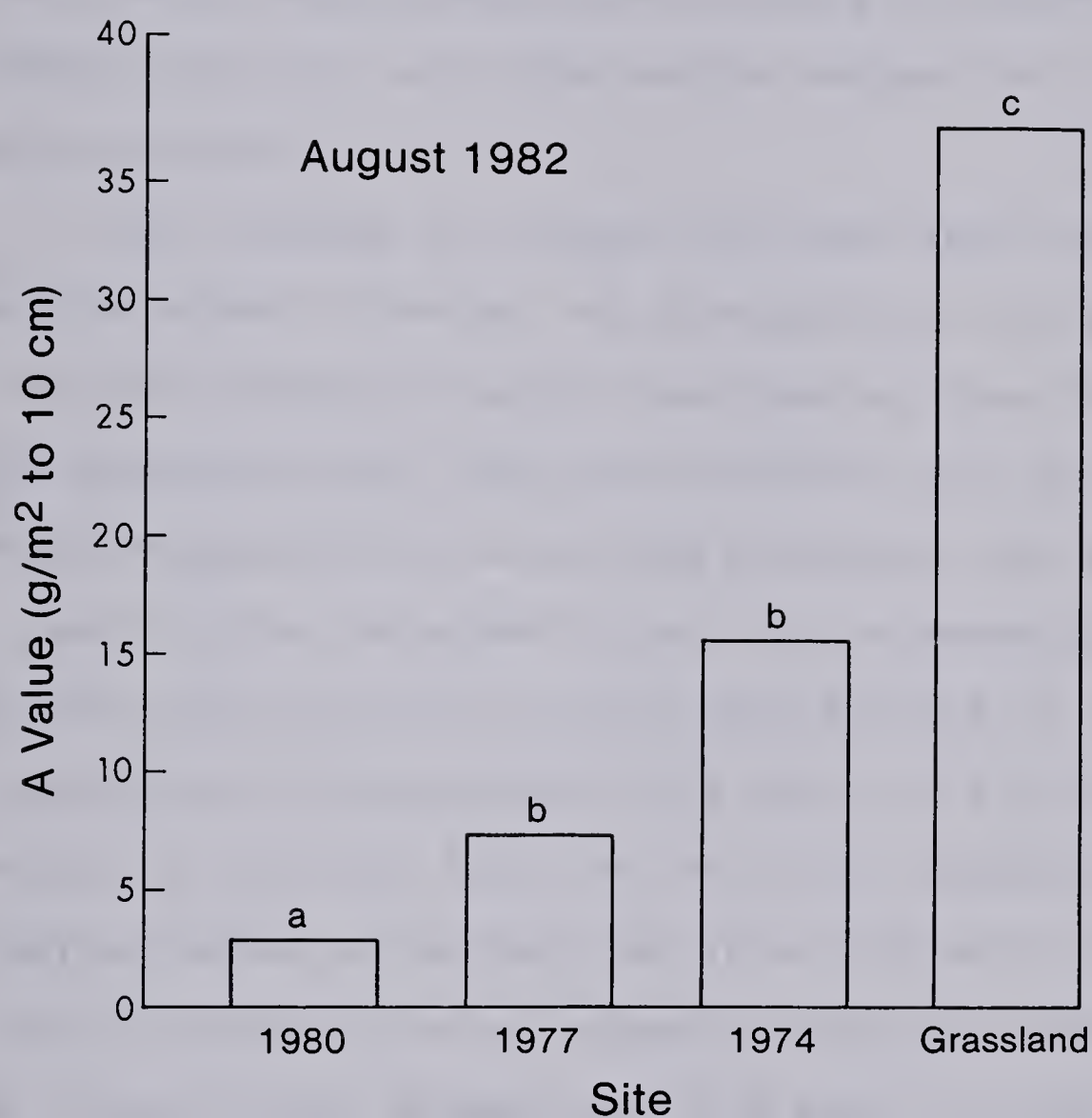


Fig. 15: Size of the active fraction of organic N in soil from the four study sites. Different letters indicate significant differences ($P \leq 0.05$) between sites.

mineralized during the first two weeks may have been due, in part, to the mineralization of microorganisms killed during sample drying prior to the incubation and, in part, to the release of adsorbed labelled N. For these reasons, the value for y which approximated most closely the NL:NT ratio of the active fraction, was taken as the average ratio measured after week two.

The increase in A value with site age indicated that an active organic fraction was developing in soils of the reclaimed sites although it was smaller than that present in the grassland soil. This is consistent with previous data which suggested that N cycling processes were transferring N to soil on the reclaimed sites. The increase in size of the active fraction with site age, paralleled an increase in cumulative N mineralized in the 1980, 1974 and grassland soils. In the soil from the 1977 site, however, there was a similar amount of active N as in soil from the 1974 site, but the capacity for net mineralization was much lower. Such an inconsistency between the 1974 and 1977 sites can be explained if the controls on the development of a N cycle in fertilized reclaimed areas are examined.

In a laboratory incubation, the net amount of N mineralized in soil is ultimately controlled by the C:N ratio of the active fraction of the organic matter, the activity of microorganisms, and their C utilization efficiency. Because it was unlikely that microorganisms were fundamentally different among the reclaimed sites, it was

expected that the average C utilization efficiency of soil microorganisms was similar among sites. The similarity in soil respiration measured in the study sites (Table 17), and the expected similarity in C utilization efficiency, suggested that microbial activity was not sufficiently variable to account for between site differences in net N mineralization. The C:N ratio of the active fraction was therefore considered to be the dominant factor affecting net N mineralization.

The very small amount of net N mineralized in the soil of the 1977 site indicated that the C:N ratio of the active fraction was higher than in soil of the other sites.

A schematic diagram summarizing the major components and flows in the reclaimed systems is shown in Figure 16. Prior to seeding and fertilization, none of these components exist, with the possible exception of some recalcitrant N from the shale. The addition of fertilizer N temporarily creates a large pool of mineral N which supports the growth of establishing vegetation. The plant shoots and a portion of the roots, senesce at the end of the growing season, transferring high C:N ratio plant material to the litter component. Fragmentation and decomposition during the following months transfers some of this material to the active organic fraction. During subsequent growing seasons, annual additions of fertilizer continue to support vigorous plant growth and litter production, and the size of the active fraction increases. As long as the annual input of

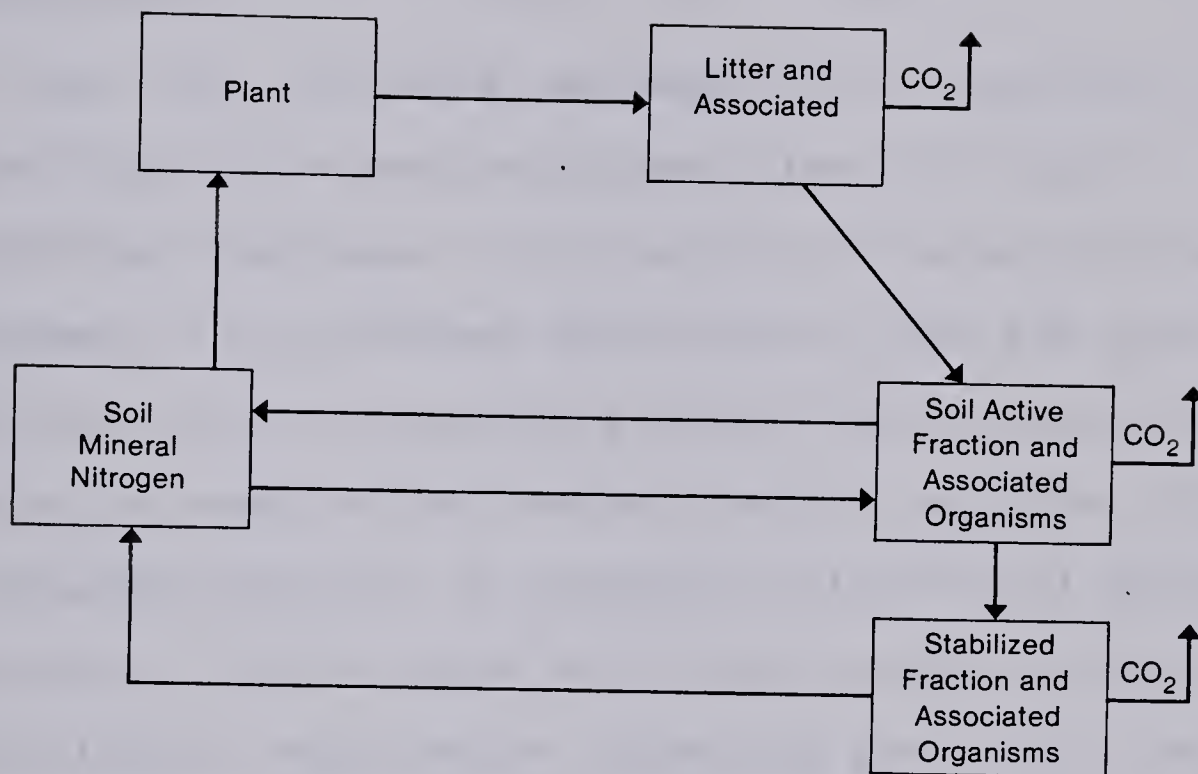


Fig. 16: Simplified and schematic diagram showing components and flows involved in N recycling in reclaimed sites. Quantities of N in each component and magnitude of N flows vary with stage of system development.

high C:N ratio material from the litter is large, relative to the size of the active fraction, the C:N ratio of the active fraction will also be high. At this time, any N mineralized from the active fraction will be re-immobilized by microorganisms or by plant growth. Fertilization supplies sufficient mineral N to maintain a high level of plant productivity.

Over time, microbial decomposition of material in the active fraction causes respiratory loss of C and a concomittant decrease in C:N ratio. As the active fraction increases in size, annual additions of high C:N ratio residues will be increasingly diluted by the lower C:N ratio material already in the active fraction, and the influence of the added material on average C:N ratio will decrease. Eventually, the C:N ratio will reach a point at which net mineralization supplies an increasing portion of the N used for plant growth.

Such development of the active fraction is consistent with the data for the reclaimed sites. On the 1980 site, the size of the litter component was small and consequently transfer of high C:N ratio material to the active fraction was also small. The capacity of microorganisms to immobilize N mineralized from the active fraction is overshadowed by the amount of N present in the soil from previous additions of fertilizer N and conditions of net mineralization occur.

On the five year old site, transfers from the litter component to the active fraction are large relative to the

size of the accumulated active fraction, raising the C:N ratio above that required for net mineralization. On the nine year old reclaimed site, fertilized only once in the last three years, the amount of N in the active fraction did not differ from the five year old site (Figure 15). On the older site, however, plant production declined from 2800 to 1800 kg/ha when fertilization was stopped in 1978 (Kaiser Resources Ltd. 1978, 1979), and litter production and inputs of high C:N ratio residue to the active fraction therefore adjusted accordingly. The additional four years of development have apparently resulted in a lower C:N ratio of the active fraction and have provided conditions which allowed net mineralization during the laboratory incubation.

The presence of stabilized or recalcitrant organic N in the soil of the reclaimed sites was described in Figure 16, but little is known about the rate of accumulation of this organic material. The decrease in NL:NT ratio observed in the grassland soil during the incubation indicated the presence of N mineralized from the recalcitrant material. No such decrease in NL:NT ratio was observed in the 1974 site although the existence of a stabilized, unlabelled fraction was indicated in an earlier section.

F. MICROBIAL BIOMASS

Soil microorganisms link the organic N and mineral N pools. The size and activity of the microbial component controls the rate of N mineralization and immobilization. In

this study, the size of the microbial biomass was estimated using the chloroform fumigation technique and biomass activity was related to soil respiration measured during a laboratory incubation.

RESULTS

The amount of CO₂ evolved from soil during a short term incubation was lowest on the 1980 site and highest on the native grassland (Table 17). No significant difference was measured in CO₂ evolution between the 1977 and 1974 soil samples.

Microbial biomass C and N as measured by the fumigation technique, were significantly higher in the grassland soil than in any of the reclaimed sites (Table 18). No significant difference in biomass C or N was measured among the reclaimed sites. The average C:N ratio measured in the biomass was 14 in the grassland soil and 24 in the 1974 site although it varied from 11 to 35 in the latter site. On the 1977 site, ratios tended to be lower than on the 1974 site except for one extremely high value in September 1982. The C:N ratio calculated for the biomass on the 1980 site was high throughout the study period and averaged 156.

The NL:NT ratios in the control and fumigated soils were highest in the 1980 site and no difference was detected between the other sites (Table 19). On the three reclaimed sites the NL:NT ratio of N mineralized from the fumigated soil was higher than that mineralized from the control.

Table 17. CO₂ evolved from soil of the four study sites during an 11 day incubation (mg C/g soil/11 days)

| SITE | CO ₂ EVOLVED |
|-----------|----------------------------------|
| 1980 | 0.37 ¹ a ² |
| 1977 | 1.22 b |
| 1974 | 1.19 b |
| GRASSLAND | 2.29 c |

1. Mean of 20 replicate soil samples collected on five sampling dates during the study period.
2. Different letters indicate significant differences ($p \leq 0.05$) between sites.

TABLE 18. BIOMASS CARBON AND NITROGEN MEASURED AT THE FOUR STUDY SITES (ug/g soil)

| SITE | JUNE 1982 | | | AUGUST 1982 | | | SEPTEMBER 1982 | | | JUNE 1983 | | | AUGUST 1982 | | |
|---------------|-----------|------|-----|-------------|------|-----|----------------|------|-----|-----------|------|-----|-------------|------|-----|
| | C | N | C/N | C | N | C/N | C | N | C/N | C | N | C/N | C | N | C/N |
| 1980 | 6581a2 | 51a | 132 | 623a | 7a | 89 | 883a | 7a | 126 | 389a | 1a | 389 | 411a | 9a | 46 |
| 1977 | 18a | 20a | 1 | 318a | 12a | 27 | 240a | 32a | 8 | 106a | 20a | 5 | 589a | 1a | 589 |
| 1974 | 355a | 33a | 11 | 1010a | 30a | 34 | 475a | 46a | 10 | 1180a | 34a | 35 | 773a | 25a | 31 |
| GRASS LAND | 7970b | 433b | 18 | 6300b | 538b | 12 | 7810b | 563b | 14 | 7330b | 650b | 11 | 7870b | 496b | 16 |

- 1. The k value used to calculate biomass C was 0.45 (Jenkinson and Ladd 1981).
- 2. Different letters indicate significant differences (p<0.05) between sites.
- 3. The k value used to calculate biomass N was 0.30 (Voroney and Paul 1984).

TABLE 19. NL:NT RATIO OF NITROGEN MINERALIZED FROM FUMIGATED AND CONTROL SOILS

| DATE | 1980 SITE | | | 1977 SITE | | | 1974 SITE | | | GRASSLAND | | |
|-----------|--------------------|-------|--|-----------|------|--|-----------|------|--|-----------|------|--|
| | CONTROL | FUM. | | CONTROL | FUM. | | CONTROL | FUM. | | CONTROL | FUM. | |
| June 1982 | 54.7a ¹ | 50.2 | | 5.7b | 1.4b | | 4.1b | 4.2b | | 2.8b | 8.9b | |
| Aug. | 43.6a | 40.9a | | 5.5b | 3.5b | | 5.6b | 3.2b | | 1.5b | 2.9b | |
| Sept. | 45.0a | 36.6a | | 6.8b | 0.6b | | 3.6b | 2.5b | | 1.1b | 1.3b | |
| June 1983 | 30.0a | 32.7a | | 10.6b | 5.6b | | 4.1b | 3.3b | | 2.8b | 3.2b | |
| Aug. | 25.3a | 26.9a | | N.D. | N.D. | | 5.0b | 2.6b | | 1.5c | 1.8b | |

1. Different letters indicated significant differences ($p \leq 0.05$) within any treatment and between sites.

Conversely, on the grassland site, the NL:NT ratio of N mineralized in the fumigated soil was consistently lower than that of the control.

DISCUSSION

The increase in CO₂ evolution with site age suggested that microbial activity was related to the size of the active organic fraction. NL:NT ratios of N mineralized from the control and fumigated soils are consistent with ratios measured in the N mineralization experiment of the previous section.

The amount of C in the microbial biomass in the native grassland soil was similar to that measured by the same method on grassland soils at Rothamsted (Jenkinson and Powlson 1976b), and biomass C:N ratio was within the range of C:N ratios reported for bacteria and fungi (McGill *et al.* 1981). The higher NL:NT ratio of N mineralized by the grassland control soil than by the fumigated soil at all sampling dates, indicated that the fumigated biomass was less highly labelled than the biomass decomposing organic material in the control. Microorganisms decompose all fractions of soil organic matter and those decomposing the highly labelled active fraction would have a higher NL:NT ratio than those decomposing the unlabelled recalcitrant fraction. The average NL:NT ratio of the N mineralized from the entire microbial biomass in the fumigated soil, would therefore be less than the ratio of the N mineralized from

the active fraction and from active organisms. The observation that the % excess of the N mineralized from the fumigated soil was similar to the % excess of the N mineralized from the active fraction in the previous study, and at least ten times greater than that measured in the total soil is consistent with this conclusion.

The amount of N in the biomass was approximately 10% of the amount of N in the active fraction in soils of the reclaimed sites and 50% of the amount of N in the active fraction in the grassland soil. The relatively large size of the biomass compared to the active fraction in the grassland is consistent with previous results which suggested that the measured biomass was associated with all fractions of the soil organic matter and not just the active fraction. It is also possible that fumigation altered the organic matter so that some organic N was mineralized and included in the biomass N measurement, resulting in an overestimate of biomass N. Fumigated samples from the native grassland filtered more slowly than control samples suggesting that such an alteration could have occurred. The relatively low biomass N in the active fraction of soils from the reclaimed sites indicates the absence of a recalcitrant fraction in these sites. However, immobilization of N by organisms decomposing high C:N ratio residue may have contributed to the low biomass N measured.

Variability in the measure of C:N ratio of the biomass among the reclaimed sites suggested that increases in

biomass N did not always parallel increases in biomass C. The higher NL:NT ratio of N mineralized from the fumigated soil than from the control soil, indicated that N from dead microbial biomass was being mineralized in soil from all sites. The flush of microbial activity commonly associated with such decomposition did not occur in over 25% of the soil samples tested from the 1977 and 1974 sites, and it is apparent that the controls on CO₂ evolution were not related only to the readily available C and N in dead microorganisms. The unrealistically high C:N ratio of the biomass on the 1980 site suggested that immobilization of N consistently resulted in an underestimate of biomass N.

The above results cannot be explained with the present knowledge of N cycling in reclaimed systems, although parallel trends in biomass C and the amount of N mineralized in the 23 week incubation (Part F) suggest a connection between high C:N ratios in the active organic fraction and variation in amounts of CO₂ evolved following fumigation. Although the fumigation technique did not provide an unequivocal measurement of microbial biomass in the reclaimed sites in this study, it did suggest that controls on microbial activity in systems not at steady-state may be more difficult to measure than in well-developed, stable systems. Further use of this technique to monitor responses of microbial activity in reclaimed soil to additions of readily available sources of C and N may further explain the processes controlling C and N cycling in fertilized

reclaimed systems.

G. NITROGEN FIXATION

The frequency of occurrence of N_2 fixing bacteria associated with plant roots and the potential contribution of asymbiotic N_2 fixation to soil N accumulation, were assessed in the four study sites.

RESULTS

No measurable amount of acetylene was reduced by the roots of any of the grass species tested under field conditions. Legumes tested under the same conditions reduced acetylene and confirmed that the method was working.

Of the plants tested in the laboratory, a smaller proportion of the plant roots fixed N_2 on the 1980 site than on the other sites (Table 20). Few consistent results were obtained between individual plants of a single species.

Root cultures which had reduced acetylene in the laboratory were plated out and an attempt was made to classify the bacteria. Isolated bacteria had characteristics of *Azotobacter* but a confirmed identification was not made.

DISCUSSION

The presence of nonsymbiotic N_2 fixing bacteria, living in association with the roots of agronomic and native grass species was confirmed in all study sites. The absence of fixation activity under field conditions indicated that the

TABLE 20. A SURVEY OF ASYMBIOTIC NITROGEN FIXERS ASSOCIATED WITH PLANT ROOTS IN THE STUDY SITES

NUMBER OF PLANTS WITH ASYMBIOTIC N₂ FIXERS/NUMBER OF PLANTS TESTED

| SPECIES | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
|-------------------------------|--------------|--------------|--------------|---------------|
| AGRONOMIC | | | | |
| <i>Agrostis scabra</i> | O/2 | | | |
| <i>Alopecurus pratensis</i> | 1/2 | | 1/1 | |
| <i>Bromus inermis</i> | | 2/3 | O/1 | |
| <i>Dactylis glomerata</i> | O/1 | 1/3 | O/1 | |
| <i>Festuca rubra</i> | O/4 | 1/2 | 3/3 | |
| <i>Phleum pratenses</i> | 1/3 | 3/3 | | |
| <i>Poa pratensis</i> | | 1/1 | 2/2 | |
| NATIVE | | | | |
| <i>Achillia millefolium</i> | | | | O/1 |
| <i>Allium cernuum</i> | | | | O/1 |
| <i>Amelanchier alnifolia</i> | | | | 1/1 |
| <i>Antennaria microphylla</i> | | | | O/1 |
| <i>Arabis holboellii</i> | | | | O/1 |
| <i>Aster conspicuus</i> | | | | 1/1 |
| <i>Epilobium</i> | | | | |
| <i>angustifolium</i> | | | | 1/1 |
| <i>Festuca idahoensis</i> | | | | O/1 |
| <i>Geranium</i> | | | | |
| <i>viscosissimum</i> | | | | O/1 |
| <i>Lomatium dissectum</i> | | | | O/1 |
| <i>Penstemon confertis</i> | | | | 1/1 |
| <i>Poa canbyi</i> | | | | 1/1 |
| <i>Poa leptocoma</i> | | | | 1/1 |
| <i>Potentilla</i> | | | | 1/1 |
| <i>pennsylvannica</i> | | | | 1/1 |
| PLANTS WITH FIXERS | | | | |
| TOTAL PLANTS TESTED | 2/12 | 8/12 | 6/8 | 7/14 |

contribution of N_2 fixation to N accumulation in the reclaimed systems was small.

A greater proportion of plants on the older study sites had N_2 fixing bacteria associated with roots than on the 1980 site. It is possible that the increased root development and soil organic matter accumulation in the old sites had enhanced the growth of N_2 fixing bacteria. Also, the relatively large mineral N pool described previously, and the small root and organic matter components in the soil of the 1980 site, may retard the establishment of such a population in young reclaimed systems.

VII. OVERALL SUMMARY AND CONCLUSIONS

Changes in the capacity of the reclaimed sites to recycle N occurred as site age increased. All of the plant and soil components were increasing in size on the two year old site, and transfers of N from plant residues to soil were small. The active fraction in the soil of this site was therefore in the initial stage of development and net mineralization of N was insufficient to maintain site productivity. Plant growth was heavily dependent on inputs of mineral N through fertilization.

Shoot, crown and root components had reached a stable maximum on the five and nine year old reclaimed sites although the latter component was probably increasing below the 10 cm sampling depth. The litter component was increasing in size but at a slower rate than on the two year old site. Annual transfers of N from litter and root components in the five and nine year old sites were calculated at 58 and 89 kg/ha, respectively, and demonstrated the increasing amount of N recycled as site age increased. Such transfers had produced pools of organic N in the soil of both sites. On the five year old site, this organic N consisted almost entirely of the active fraction. Although this fraction mineralized sufficient N to supply a major portion of the N utilized by the plants, the high C:N ratio of the active fraction suggested that competition for mineral N was intense in this system. Termination of fertilization at this stage of development would therefore

be expected to result in a reduction in plant productivity.

Soil organic N on the nine year old site also consisted mainly of the active fraction but the development of a stabilized fraction was also evident. Absence of fertilizer inputs to this system in the previous two out of three years, prior to this study, had initially resulted in a decrease in plant productivity and a subsequent readjustment of the other components. The C:N ratio of the active fraction was therefore lower, and net mineralization higher, than in the five year site. The proportion of N utilized by the plant which was derived from the labelled N was lower than at the five year site demonstrating that the capacity of reclaimed systems to mineralize N increases with site age.

Most of the N mineralized in the reclaimed sites came from the active fraction of soil organic matter, and the stability of this fraction is a major control on the ability of each system to recycle N. It is not known if the amount of N mineralized by the active fraction will be reduced over the long term due to transfer to the recalcitrant fraction.

The high elevation native grassland examined in this study, was considered to be in a steady-state condition which would eventually be attained by the reclaimed systems. Knowledge of the size and turnover of soil and plant components on this self-sustaining site, and the capacity of the soil to mineralize N, therefore provided a basis on which to assess the ability of the reclaimed sites to

recycle N and sustain plant productivity.

Shoot and crown components were of similar size, and the root component smaller, on the oldest reclaimed systems compared to the native grassland. The litter component and the active organic fraction each contained approximately twice as much N as those components on the nine year site. The amount of N transferred to the soil from the roots and litter was estimated at 123 kg/ha annually, a quantity comparable with N flow in the nine year old site. The major difference between the N cycle of the oldest reclaimed site and that of the native grassland, was the presence of a large recalcitrant fraction of organic N in the soil of the latter site. Although rate of N mineralization by such a fraction is slow, the cumulative amount of mineral N contributed a significant proportion to total N mineralized in the system. The absence of a sizeable recalcitrant component in the reclaimed sites indicated that the capacity of these systems to mineralize N is lower than in the native grassland. The long term ability of the reclaimed systems to provide sufficient mineral N to maintain plant productivity may depend on the development of a stabilized pool of organic N in addition to the active pool.

Three general conclusions about N cycling in high elevation reclaimed mine spoil may be drawn from this work:

1. Nitrogen in surface litter and in roots has a turnover time of about one and two years respectively and, over the long term, accumulation of N within reclaimed systems is in the soil.

2. The size of the soil organic N pool increases with site age under current management practices and, after at least nine years, mineralizes sufficient N to maintain plant productivity without further inputs of fertilizer N.
3. Depletion of the active fraction due to transfers to the recalcitrant fraction may, in the long term, control the stability of reclaimed systems.

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IX. APPENDICES

APPENDIX I.1 HARMER RIDGE WEATHER DATA AVERAGE FROM 1976 TO 1980

| <u>MONTH</u> | <u>TEMPERATURE (°C)</u> | | | <u>PRECIPITATION (mm)</u> | |
|--------------|-------------------------|--------------------|--------------------|---------------------------|-------|
| | Average Maximum | Average Minimum | Extreme Maximum | Extreme Minimum | |
| January | -8.5 | -15.4 | -0.2 | -26.7 | 82.1 |
| February | -7.1 | -11.7 | 1.5 | -22.2 | 94.6 |
| March | -2.2 | -10.2 | 4.9 | -22.5 | 57.7 |
| April | 4.4 | -4.3 | 14.3 | -13.2 | 42.9 |
| May | 8.6 | -0.6 | 18.3 | -4.0 | 63.6 |
| June | 13.7 | 3.5 | 21.5 | -3.2 | 27.6 |
| July | 17.2 | 6.5 | 24.4 | -0.6 | 52.6 |
| August | 15.4 | 5.4 | 22.9 | 0.6 | 59.3 |
| September | 12.2 | 2.8 | 19.4 | -3.2 | 54.9 |
| October | 5.8 | -2.4 | 15.2 | -10.6 | 27.2 |
| November | -3.9 | -10.3 | 4.2 | -22.1 | 54.4 |
| December | -7.1 | -13.2 | 0.3 | -25.9 | 133.1 |

APPENDIX I.2 HARMER RIDGE WEATHER DATA 1982-1983

| MONTH | TEMPERATURE(°C) | | | PRECIPITATION(mm) | |
|-----------|--------------------|--------------------|--------------------|--------------------|-------|
| | Average Maximum | Average Minimum | Extreme Maximum | Extreme Minimum | |
| 1982 | | | | | |
| January | -8.0 | -16.7 | -2.0 | -46.0 | 90.5 |
| February | -6.0 | -13.0 | 4.5 | -26.0 | 109.5 |
| March | -1.4 | -9.1 | 9.0 | 14.5 | 80.8 |
| April | 1.5 | -7.2 | 11.0 | -18.0 | 33.5 |
| May | 9.9 | 0.0 | 17.0 | -11.0 | 23.7 |
| June | 16.7 | 7.7 | 23.0 | -1.0 | 73.4 |
| July | 16.5 | 8.0 | 26.0 | 0.5 | 20.3 |
| August | 17.4 | 7.5 | 23.0 | 2.0 | 36.5 |
| September | N.D. | N.D. | N.D. | N.D. | N.D. |
| October | N.D. | N.D. | N.D. | N.D. | N.D. |
| November | -4.7 | -10.7 | 0.0 | -18.0 | 60.5 |
| December | -6.0 | -11.3 | 0.5 | -16.5 | 57.3 |
| 1983 | | | | | |
| January | -2.1 | -7.2 | 2.5 | -11.0 | 63.5 |
| February | -1.5 | -5.7 | 5.5 | -14.0 | 49.3 |
| March | N.D. | N.D. | N.D. | N.D. | N.D. |
| April | 5.5 | -3.2 | 13.0 | -11.5 | 44.0 |
| May | 11.8 | 2.0 | 24.0 | -3.0 | 21.0 |
| June | 15.1 | 4.6 | 19.5 | 0.5 | 41.7 |
| July | 16.4 | 6.3 | 24.0 | 2.0 | 74.7 |
| August | 22.4 | 10.2 | 27.0 | 4.5 | 23.3 |
| September | 14.6 | 1.2 | 22.5 | -7.0 | 32.9 |
| October | 9.5 | -1.1 | 19.0 | -6.5 | 23.4 |
| November | -1.4 | -5.8 | 11.5 | -17.5 | 55.7 |

APPENDIX II. COVER OF PLANT SPECIES IN THE FOUR STUDY SITES(%)

| SPECIES | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
|---------------------------------|----------------|--------------|--------------|---------------|
| AGRONOMIC | | | | |
| <i>Agrostis scabra</i> | 3 ¹ | 0 | 0 | 0 |
| <i>Bromus inermis</i> | 0 | 0 | 19 | 0 |
| <i>Dactylis glomerata</i> | 0 | 38 | 0 | 0 |
| <i>Festuca rubra</i> | 5 | 40 | 45 | 0 |
| <i>Medicago sativa</i> | 0 | 1 | 4 | 0 |
| <i>Phleum pratense</i> | 13 | 11 | 13 | 0 |
| <i>Poa canadensis</i> | 14 | 3 | 0 | 0 |
| <i>Trifolium hybridum</i> | 10 | 0 | 1 | 0 |
| NATIVE | | | | |
| <i>Achillia millefolium</i> | 0 | 0 | 0 | 3 |
| <i>Amelanchier alnifolia</i> | 0 | 0 | 0 | 4 |
| <i>Antenaria mycrophilum</i> | 0 | 0 | 0 | 1 |
| <i>Aster conspicuus</i> | 0 | 0 | 0 | 18 |
| <i>Epilobium angustifolium</i> | 0 | 0 | 0 | 3 |
| <i>Festuca idahoensis</i> | 0 | 0 | 0 | 15 |
| <i>Geranium viscosissimum</i> | 0 | 0 | 0 | 1 |
| <i>Lomatium dissectum</i> | 0 | 0 | 0 | 5 |
| <i>Lupinus seriseus</i> | 0 | 0 | 0 | 8 |
| <i>Penstemon confertis</i> | 0 | 0 | 0 | 1 |
| <i>Poa canbyi</i> | 0 | 0 | 0 | 21 |
| <i>Poa leptocoma</i> | 0 | 0 | 0 | 1 |
| <i>Potentilla pennsylvanica</i> | 0 | 0 | 0 | 3 |

1. Each value is an average of four replicates.

Appendix III. Natural abundance of plant materials and soil

| COMPONENT | 1980 SITE | 1977 SITE | 1974 SITE | GRASS LAND |
|-----------|---------------------|--------------|--------------|---------------|
| GRASS | 0.3666 ¹ | 0.3662 | 0.3654 | 0.3654 |
| LITTER | 0.3663 | 0.3668 | 0.3686 | 0.3685 |
| ROOTS | 0.3778 | 0.3671 | 0.3656 | 0.3645 |
| SOIL | 0.3672 | 0.3703 | 0.3721 | 0.3668 |

1. Each value an average of three replicates.

APPENDIX IV.1 BIOMASS OF SHOOTS, LEGUMES AND LITTER (g/m²)

| SITE | <u>SHOOTS</u> | | | | <u>LEGUMES</u> | | | | <u>LITTER</u> | | | |
|----------------|-------------------------|-------------|-------------|------------|----------------|-----------|------------|------------|---------------|------------|--------------------------|-------------|
| | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 | June 1982 | Aug. 1982 | Sept. 1982 | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 |
| 1980 | 35 ¹ (13) | 58 (10) | 115 (7) | 18 (2) | 64 (7) | 3 (3) | 14 (11) | 6 (4) | 3 (1) | 70 (24) | 4 (1) | 58 (10) |
| 1977 | 98 (8) | 159 (11) | 195 (20) | 19 (2) | 89 (4) | 0 (0) | 0 (0) | 0 (0) | 1 (1) | 0 (0) | 205 (31) | 236 (15) |
| 1974 | 43 (10) | 179 (39) | 215 (55) | 22 (5) | 130 (38) | 1 (1) | 7 (4) | 8 (8) | 0 (0) | 0 (0) | 231 (28) | 292 (31) |
| Grass- land | 124 (157) | 168 (13) | 182 (26) | 65 (11) | 175 (11) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 372 (34) | 204 (26) |
| | | | | | | | | | | | Highly decomposed litter | |
| | | | | | | | | | | | 206 (14) | 91 (13) |
| | | | | | | | | | | | 242 (46) | 211 (22) |

1. Mean of four replicates
(standard error)

Appendix IV.2 BIOMASS OF CROWNS, COARSE ROOTS AND FINE ROOTS (g/m² to 10 cm)

| SITE | CROWNS | | | | COARSE ROOTS | | | | FINE ROOTS | | | | | | |
|----------------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|---------------|--------------|--------------|
| | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 |
| 1980 | 84 (16) | 205 (25) | 111 (15) | 126 (39) | 147 (41) | 95 (22) | 182 (19) | 268 (29) | 122 (27) | 122 (32) | 15 (1) | 24 (2) | 47 (13) | 39 (7) | 13 (4) |
| 1977 | 354 (60) | 693 (112) | 229 (29) | 242 (71) | 556 (148) | 487 (43) | 516 (50) | 249 (38) | 443 (44) | 441 (24) | 306 (36) | 607 (128) | 348 (149) | 439 (58) | 491 (80) |
| 1974 | 438 (137) | 240 (53) | 283 (97) | 445 (74) | 214 (69) | 336 (42) | 743 (184) | 435 (104) | 458 (116) | 451 (133) | 123 (5) | 635 (64) | 291 (108) | 460 (48) | 306 (53) |
| Grass- land | 273 (85) | 193 (88) | 94 (28) | 262 (93) | 174 (34) | 338 (44) | 602 (162) | 394 (100) | 280 (94) | 367 (71) | 190 (49) | 386 (33) | 259 (65) | 578 (83) | 625 (120) |

1. Mean of four replicates
(standard error)

Appendix V. Nitrogen-free medium'

| | |
|---------|---|
| 5 g | malic acid |
| 4 g | KOH |
| 0.5 g | K ₂ HPO ₄ |
| 0.2 g | MgSO ₄ *7H ₂ O |
| 0.1 | NaCl |
| 0.02 g | CaCl |
| 0.5 g | FeSO ₄ *7H ₂ O |
| 0.002 g | NaMoO ₄ *2H ₂ O |
| 2 ml | 0.5% alcoholic solution bromothymol blue |
| 0.01 g | MnSO ₄ *H ₂ O |

Make up to 1 l volume, adjust pH to 6.8 with NaOH
Add 1.75 g agar.

1. Described by Dobereiner *et al* (1976).

APPENDIX VI.1 CHEMICAL AND PHYSICAL PROPERTIES OF SOIL FROM THE STUDY SITES

| SITE | CEC (mol/kg) | pH 2:1 | PARTICLE SIZE DISTRIBUTION | | | COARSE FRAGMENTS (%) | BULK DENSITY Mg/m ³ |
|----------------|--------------------|-----------|----------------------------|-------------|-------------|----------------------------|--------------------------------------|
| | | | sand (%) | silt (%) | clay (%) | | |
| 1980 | 0.082 ¹ | 7.1 | 41 | 37 | 21 | 77 | 2.2 |
| 1977 | 0.119 | 7.0 | 37 | 37 | 24 | 79 | 2.2 |
| 1974 | 0.137 | 6.5 | 24 | 47 | 27 | 72 | 1.9 |
| GRASS- LAND | 0.433 | 6.7 | 15 | 45 | 30 | 27 | 1.4 |

1. Each value an average of four replicates.

APPENDIX VI.2 NITROGEN CONCENTRATION OF SHOOTS, CROWNS AND LITTER(%)

| SITE | SHOOTS | | | | | | CROWNS | | | | | | LITTER | | | |
|------------|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|--|
| | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 | |
| 1980 | 1.54 ¹ (0.18) | 0.72 (0.07) | 0.81 (0.03) | 1.92 (0.15) | 0.55 (0.01) | 0.80 (0.09) | 0.60 (0.01) | 0.65 (0.03) | 0.76 (0.08) | 0.61 (0.11) | 1.07 (0.08) | 0.86 (0.05) | 1.18 (0.06) | 0.78 (0.03) | 0.94 (0.08) | |
| 1977 | 1.99 (0.08) | 1.09 (0.01) | 0.77 (0.03) | 1.62 (0.03) | 0.68 (0.03) | 0.76 (0.02) | 0.70 (0.02) | 0.64 (0.04) | 0.78 (0.03) | 0.64 (0.02) | 0.91 (0.06) | 0.85 (0.04) | 0.86 (0.04) | 0.85 (0.08) | 0.98 (0.04) | |
| 1974 | 2.73 (0.11) | 0.98 (0.08) | 0.88 (0.15) | 1.97 (0.07) | 0.87 (0.03) | 0.96 (0.03) | 0.96 (0.11) | 1.00 (0.11) | 0.90 (0.07) | 0.90 (0.17) | 1.20 (0.15) | 1.13 (0.07) | 1.26 (0.16) | 1.01 (0.04) | 1.11 (0.06) | |
| Grass-land | 2.26 (0.11) | 1.02 (0.06) | 0.95 (0.02) | 2.63 (0.16) | 1.24 (0.08) | 1.32 (0.09) | 1.30 (0.06) | 1.53 (0.66) | 1.27 (0.12) | 1.24 (0.13) | 1.05 (0.04) | 1.18 (0.03) | 1.46 (0.08) | 1.01 (0.04) | 1.22 (0.07) | |

1. Mean of four replicates.
(standard error)

APPENDIX VI.3 NITROGEN CONCENTRATION IN COARSE AND FINE ROOTS AND SOIL(%)

| SITE | COARSE ROOTS | | | | | FINE ROOTS | | | | | SOIL | | | |
|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 | Aug. 1983 | June 1982 | Aug. 1982 | Sept. 1982 | June 1983 |
| 1980 | 0.67 (0.03) | 0.58 (0.02) | 0.52 (0.02) | 0.52 (0.02) | 0.55 (0.05) | 0.66 (0.05) | 0.74 (0.09) | 0.61 (0.04) | 0.72 (0.04) | 0.70 (0.06) | 0.24 (0.01) | 0.26 (0.03) | 0.21 (0.03) | 0.21 (0.01) |
| 1977 | 0.63 (0.03) | 0.57 (0.01) | 0.43 (0.02) | 0.59 (0.03) | 0.52 (0.03) | 0.72 (0.02) | 0.59 (0.02) | 0.50 (0.03) | 0.76 (0.02) | 0.68 (0.04) | 0.19 (0.01) | 0.18 (0.01) | 0.18 (0.01) | 0.20 (0.01) |
| 1974 | 0.84 (0.07) | 0.72 (0.07) | 0.54 (0.05) | 0.60 (0.08) | 0.57 (0.06) | 0.83 (0.04) | 0.69 (0.03) | 0.69 (0.05) | 0.76 (0.09) | 0.75 (0.04) | 0.16 (0.01) | 0.16 (0.02) | 0.18 (0.02) | 0.18 (0.01) |
| Grass land | 1.20 (0.10) | 1.22 (0.05) | 1.15 (0.05) | 1.07 (0.02) | 0.86 (0.07) | 1.51 (0.12) | 1.31 (0.08) | 1.50 (0.07) | 1.36 (0.05) | 1.29 (0.02) | 0.53 (0.09) | 0.61 (0.02) | 0.58 (0.08) | 0.50 (0.01) |

1. Mean of four replicates.
(standard error)

APPENDIX VII. LOSSES OF TOTAL AND LABELLED NITROGEN FROM LITTER AT EACH SAMPLING INTERVAL (mg/m²)

| SITE | JUN 1982-AUG 1982 | | AUG 1982-SEPT 1982 | | SEPT 1982-JUN 1983 | | JUN 1983-AUG 1983 | |
|----------------|-------------------|------|--------------------|-------|--------------------|--------|-------------------|-------|
| | NL | NT | NL | NT | NL | NT | NL | NT |
| 1980 | A | +8 | +130 | -165 | -252 | -284 | +18 | -96 |
| | B | -122 | -387 | -125 | -300 | -225 | 0 | -271 |
| | C | +22 | +63 | -88 | -53 | -86 | -144 | -165 |
| | D | +13 | +93 | -73 | -356 | -425 | -46 | +96 |
| 1977 | A | 0 | -361 | -1740 | -29 | -152 | -126 | +62 |
| | B | -211 | -666 | -2000 | -337 | -511 | 0 | +104 |
| | C | -90 | -213 | -601 | +33 | -655 | 0 | +421 |
| | D | 0 | +434 | -1230 | -262 | -1850 | 5 | +993 |
| 1974 | A | 0 | -445 | -1380 | +306 | +983 | -210 | -579 |
| | B | -27 | -501 | -2290 | +148 | +218 | -157 | -321 |
| | C | -85 | -3080 | +816 | -578 | -5060 | 0 | +353 |
| | D | -200 | +1410 | -3570 | -52 | -1560 | 0 | +3140 |
| GRASSA LAND | A | -188 | -2480 | +3450 | -488 | -10540 | -74 | +2160 |
| | B | -830 | -2780 | +1560 | -271 | -5550 | +61 | +4650 |
| | C | -102 | -4540 | +999 | -243 | -5900 | -168 | +2190 |
| | D | -9 | +282 | -488 | -269 | -4420 | -168 | +1770 |

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